Microbiota Intestinal e sua Dinâmica Relação com o Hospedeiro

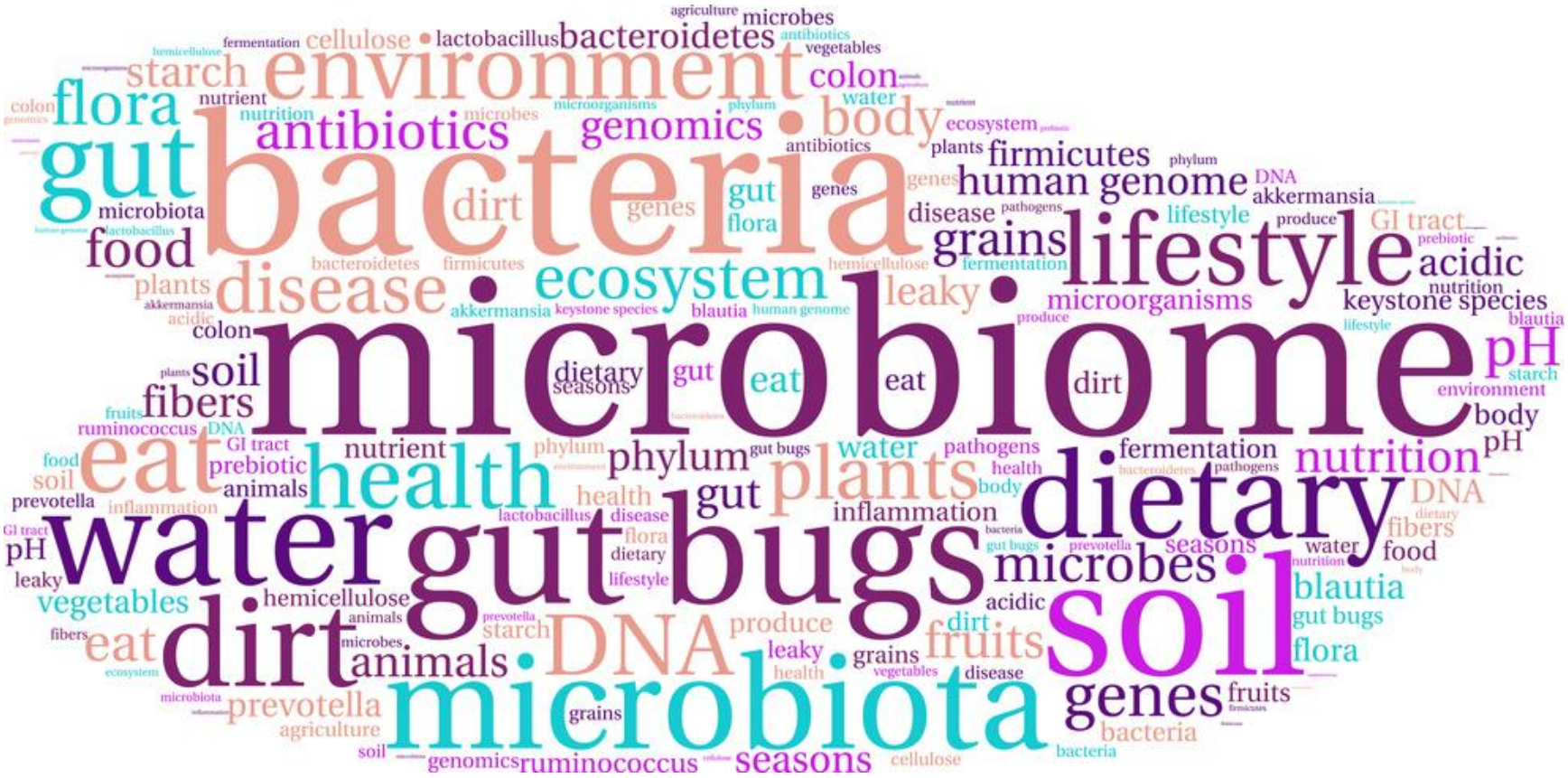


XV Congresso CBNA Pet

Dr. Maria R. C. de Godoy
Assistant Professor
Department of Animal Sciences

ILLINOIS
UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

Microbioma


microbiome
bacteria
gut
environment
microbiota
dirt
water
gut bugs
soil
dietary
plants
health
eat
lifestyle
disease
ecosystem
food
flora
antibiotics
genomics
body
starch
nutrient
nutrition
microbes
bacteroidetes
lactobacillus
cellulose
fermentation
agriculture
colon
genes
human genome
firmicutes
grains
leaky
microorganisms
keystone species
blautia
acidic
pH
fibers
DNA
prebiotic
GI tract
inflammation
vegetables
hemicellulose
prevotella
ruminococcus
seasons
fruits
produce
pathogens
akkermansia
bacteria
ruminococcus
genomics
soil
dirt
microbiota
eat
water
gut
bugs
dietary
microbes
blautia
flora
genes
fruits
DNA
animals
prevotella
agriculture
grains
seasons
bacteria
cellulose

Microbioma

- **TEMA ATUAL!!!**

- PubMed: “microbiome”
  - 2011-2016 = > 18.000 resultados
  - 1956-2016 = ~ 23.000 resultados



Microbioma

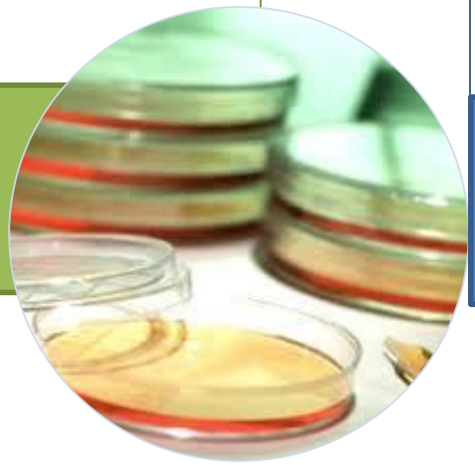
- **TEMA ATUAL!!!**

- PubMed:
  - Microbiome, feline, nutrition = 24 resultados
  - Microbiome, canine, nutrition = 34 resultados

Avanços Tecnológicos

- Pequeno número de bactérias cultivadas
- Incompleta identificação de microorganismos
- Inexistência da avaliação da função microbiana

Passado



- Disponibilidade de várias técnicas moleculares
- Custo mais accessível
- Desenvolvimento de plataformas para análise de dados e bioinformática

Atual



Avanços Tecnológicos

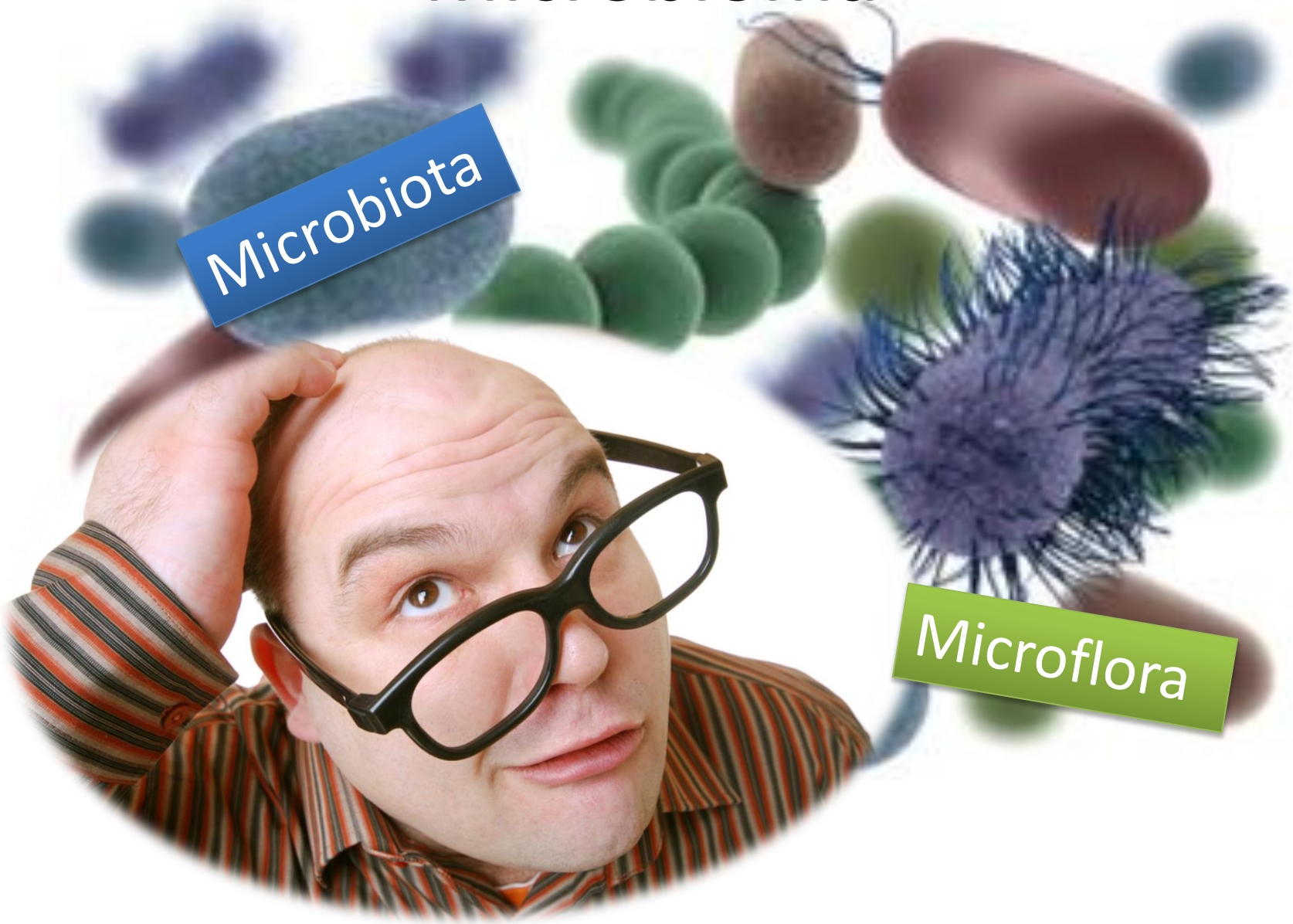


- Identificação mais efetiva da microbiota
- Investigação da função microbiana

Microbioma



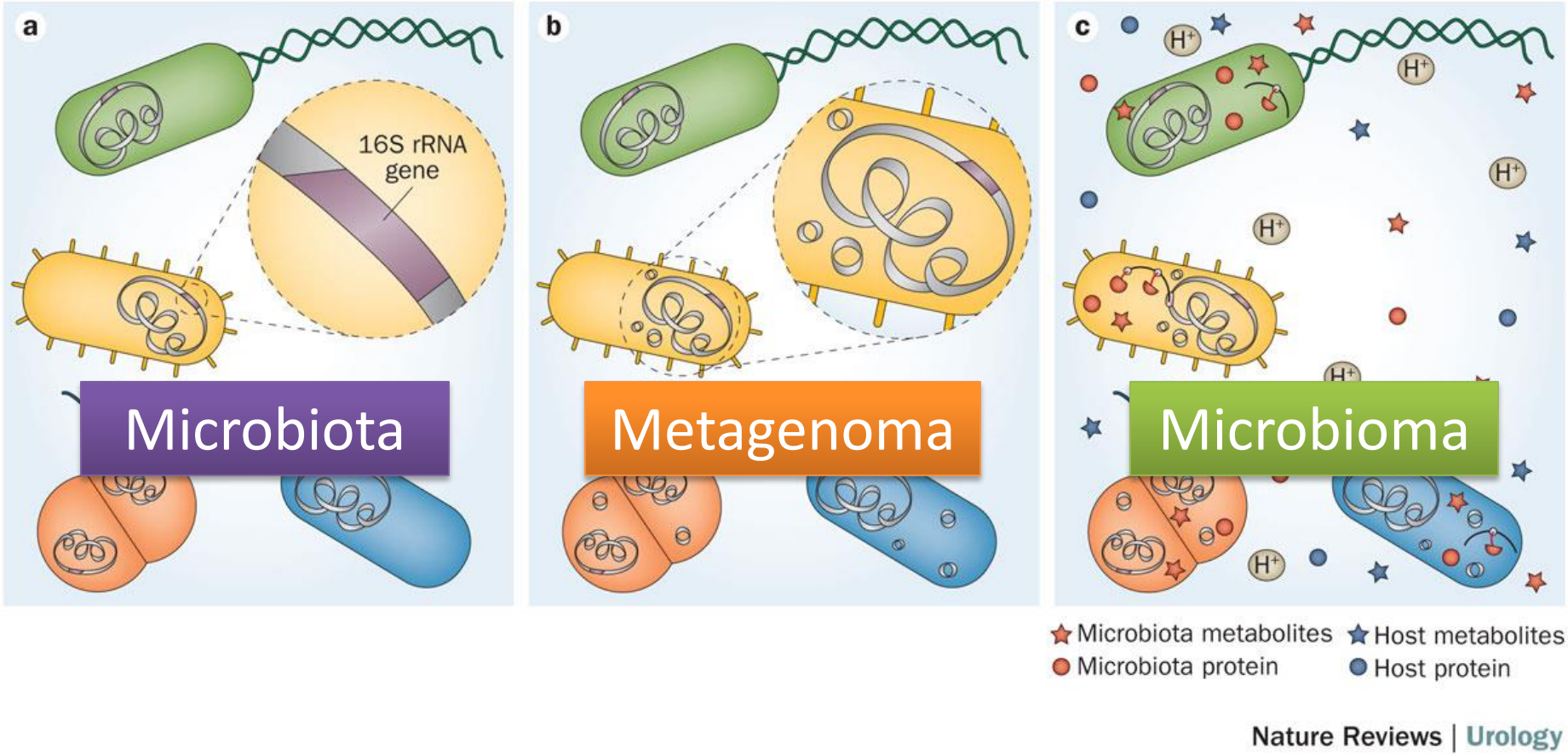
Microbioma



Microbioma

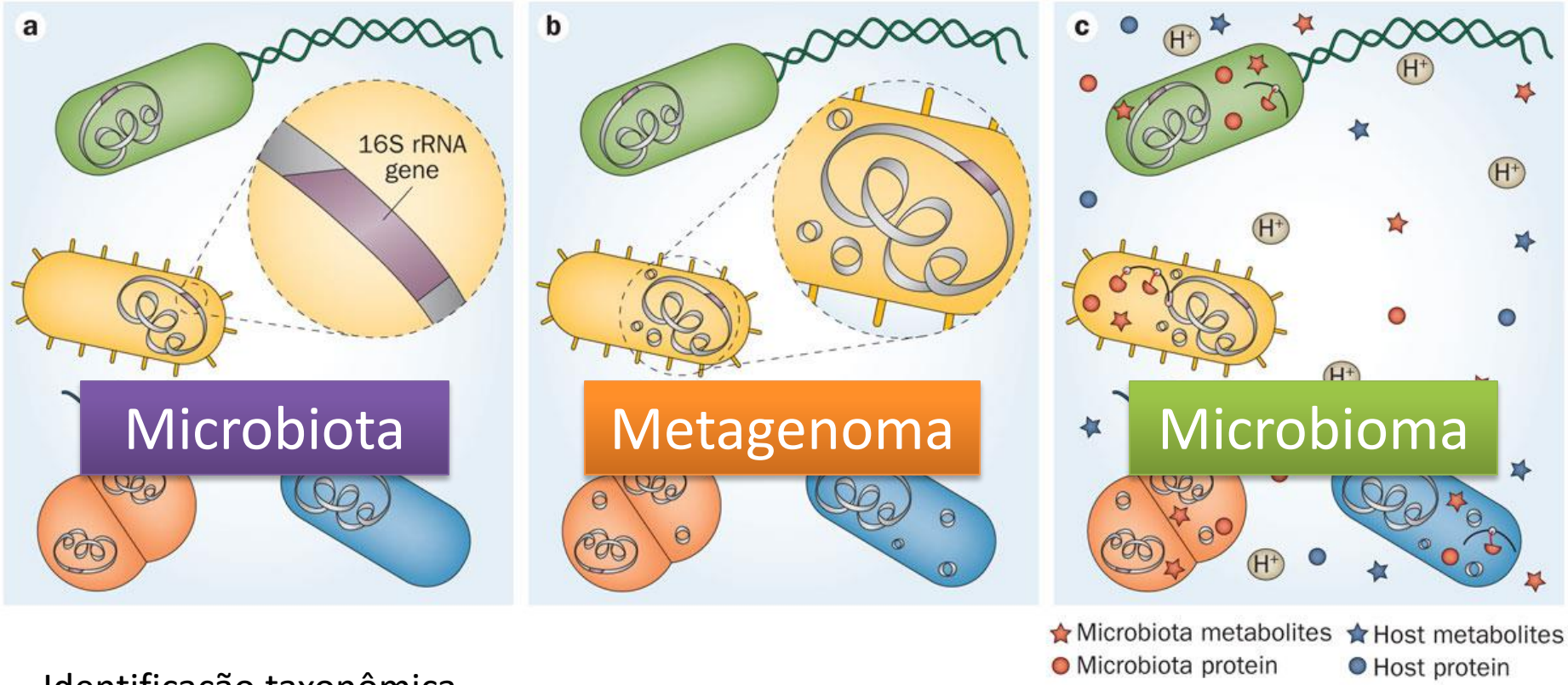


Terminologias



Whiteside et al., 2015

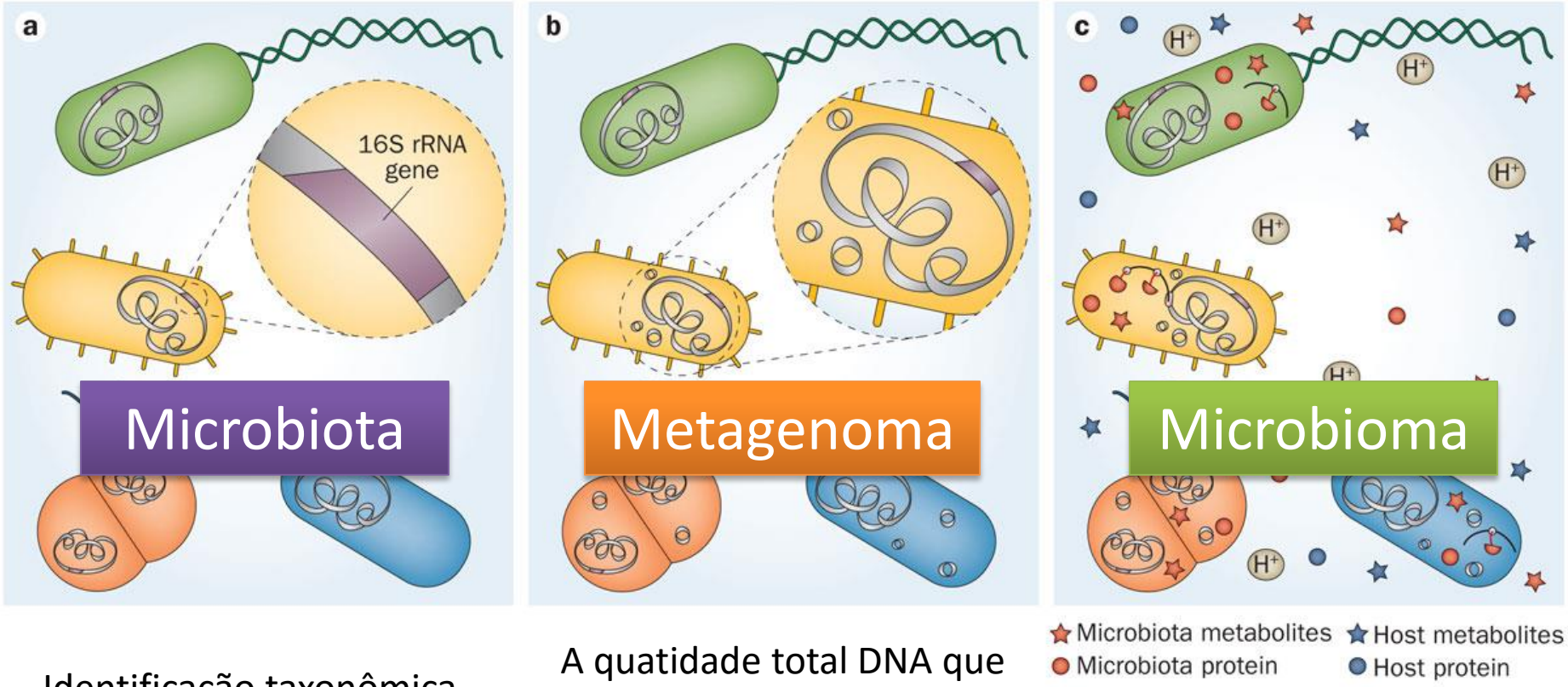
Terminologias



Identificação taxonômica dos microorganismos em um ambiente. Geralmente usando sequenciamento 16S rRNA gene

Whiteside et al., 2015

Terminologias

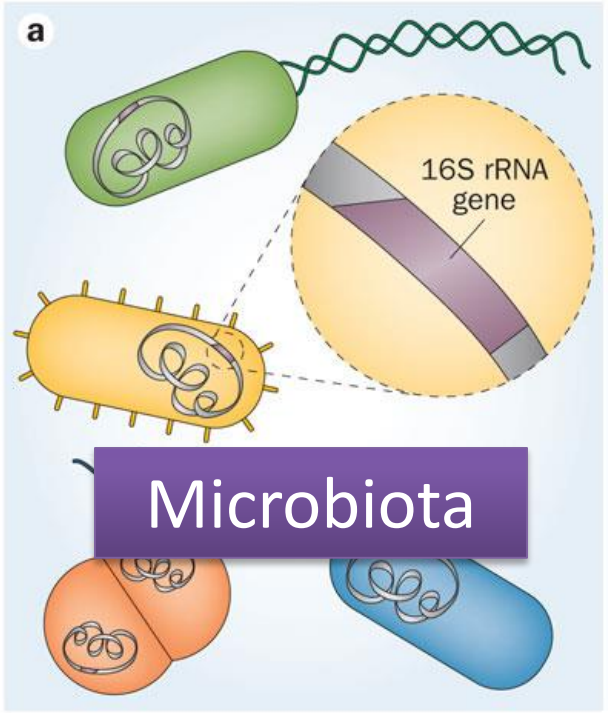


Identificação taxonômica dos microorganismos em um ambiente. Geralmente usando sequenciamento 16S rRNA gene

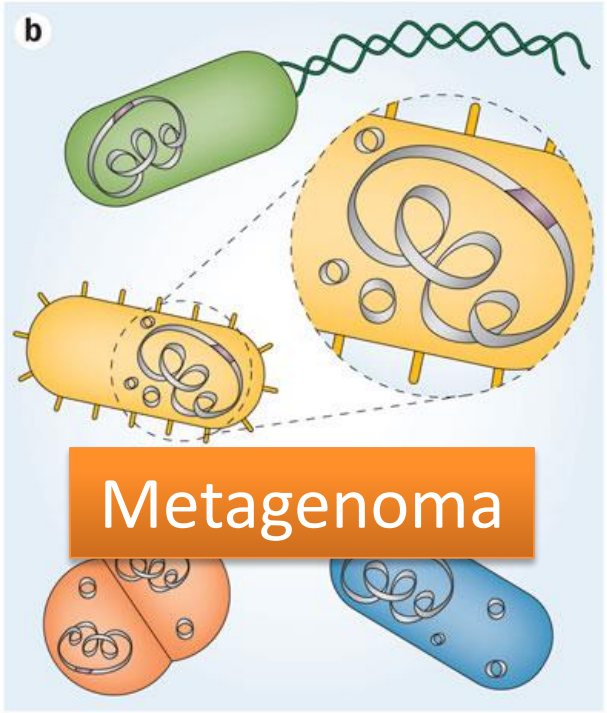
A quatidade total DNA que pode ser extraída de um determinado ambiente. Pode utilizar sequenciamente 16S rRNA ou shotgun/ WGS

Whiteside et al., 2015

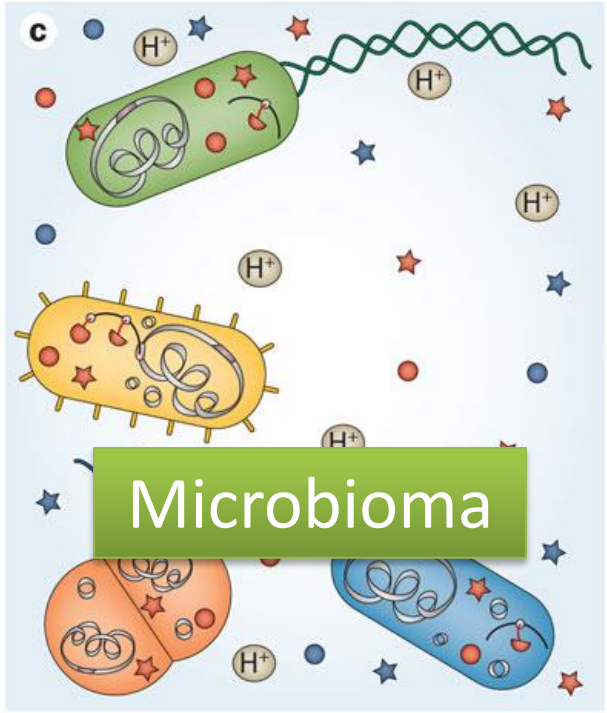
Terminologias



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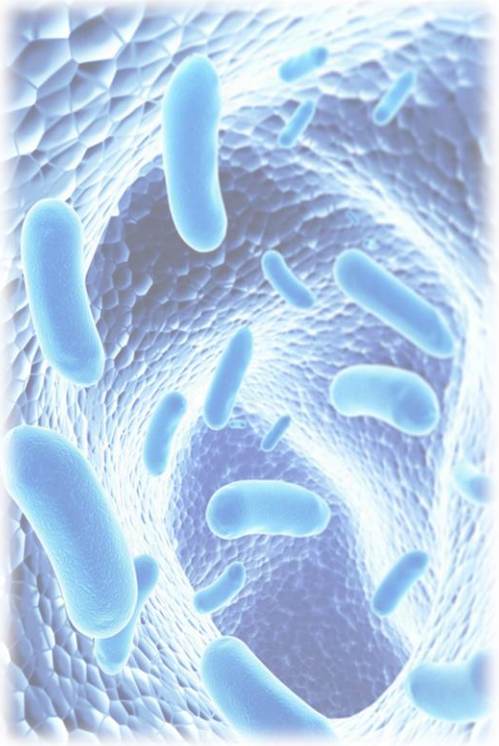


A totalidade dos microorganismos e seus materiais genéticos (potencial função) em um ambiente. Geralmente determinado por shotgun/ WGS.

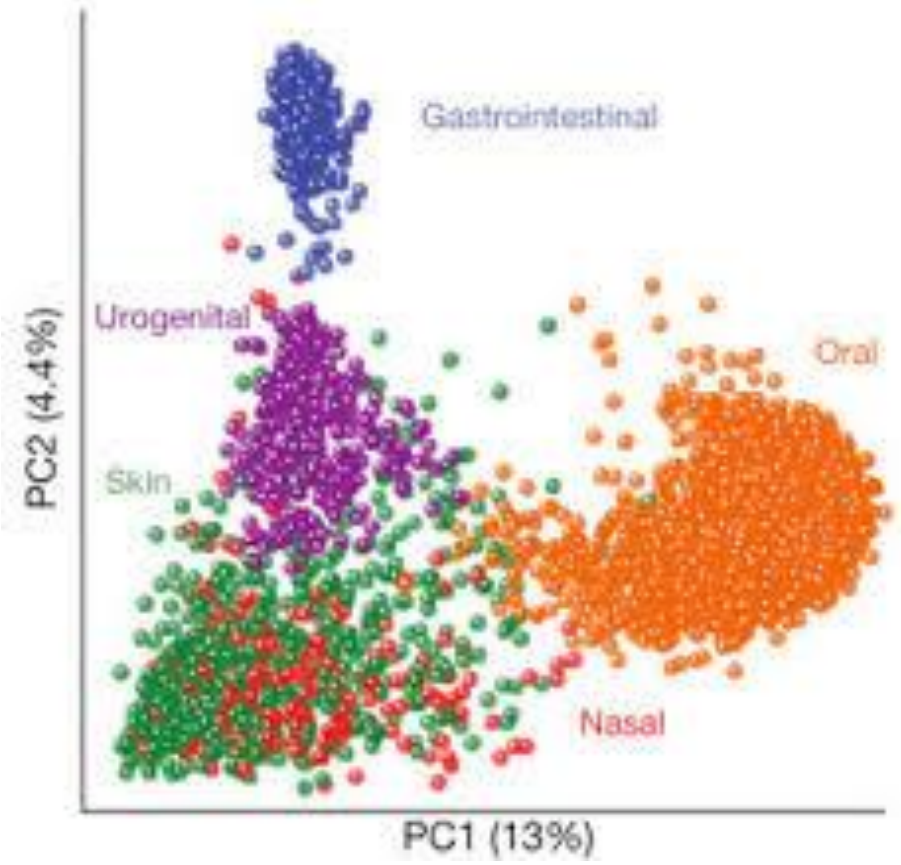
Whiteside et al., 2015

Microbioma

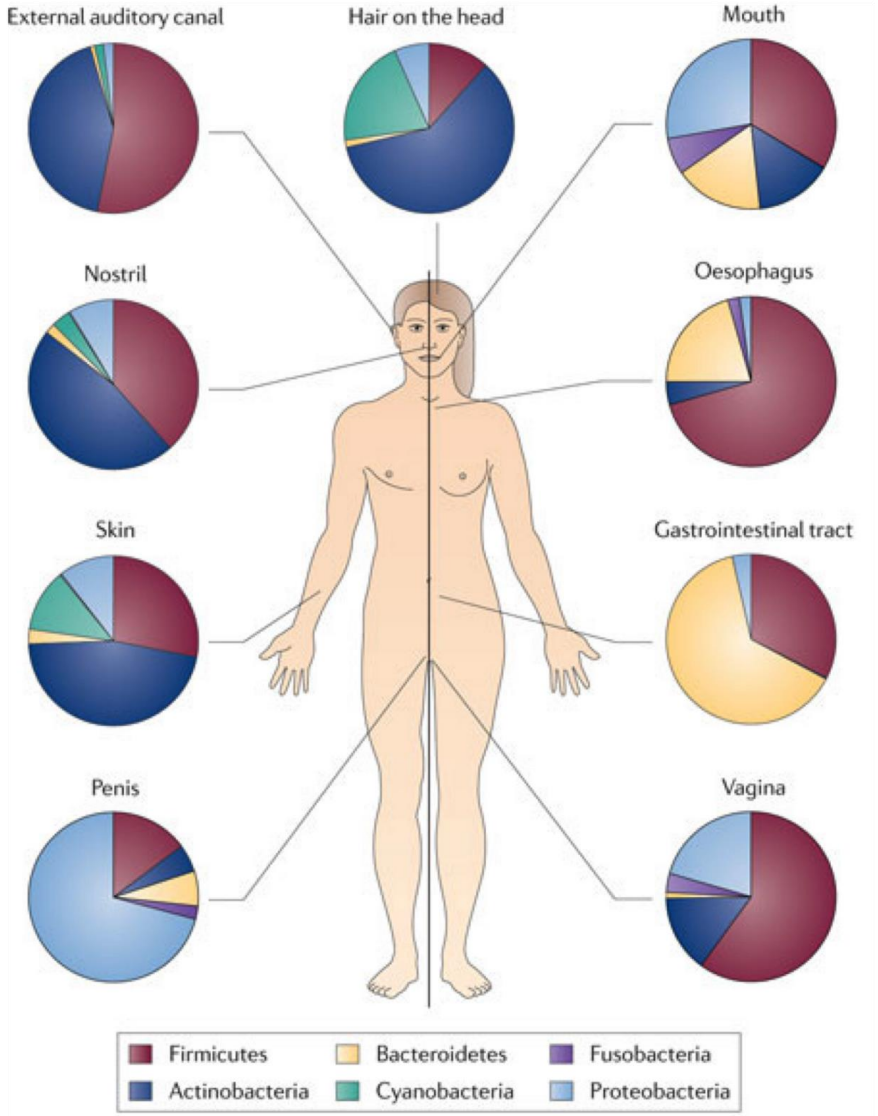
- Ecosistema complexo e diversificado
  - Bactéria
  - Arquea
  - Fungos
  - Vírus
    - Variação individual e entre indivíduos



Diversidade da Microbiota

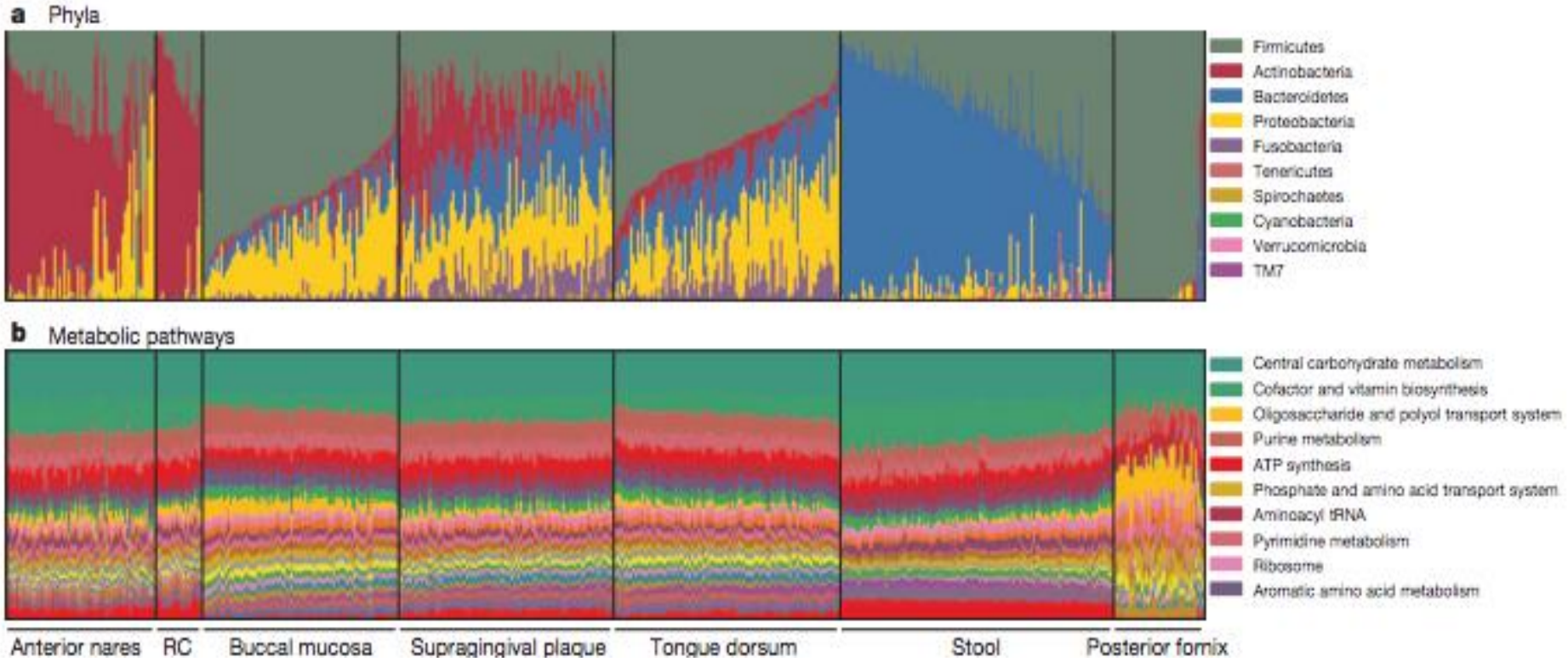


HMP Consortium, 2012



Spor et al., 2011

Diversidade da Microbiota



HMP Consortium, 2012

Diversidade da Microbiota

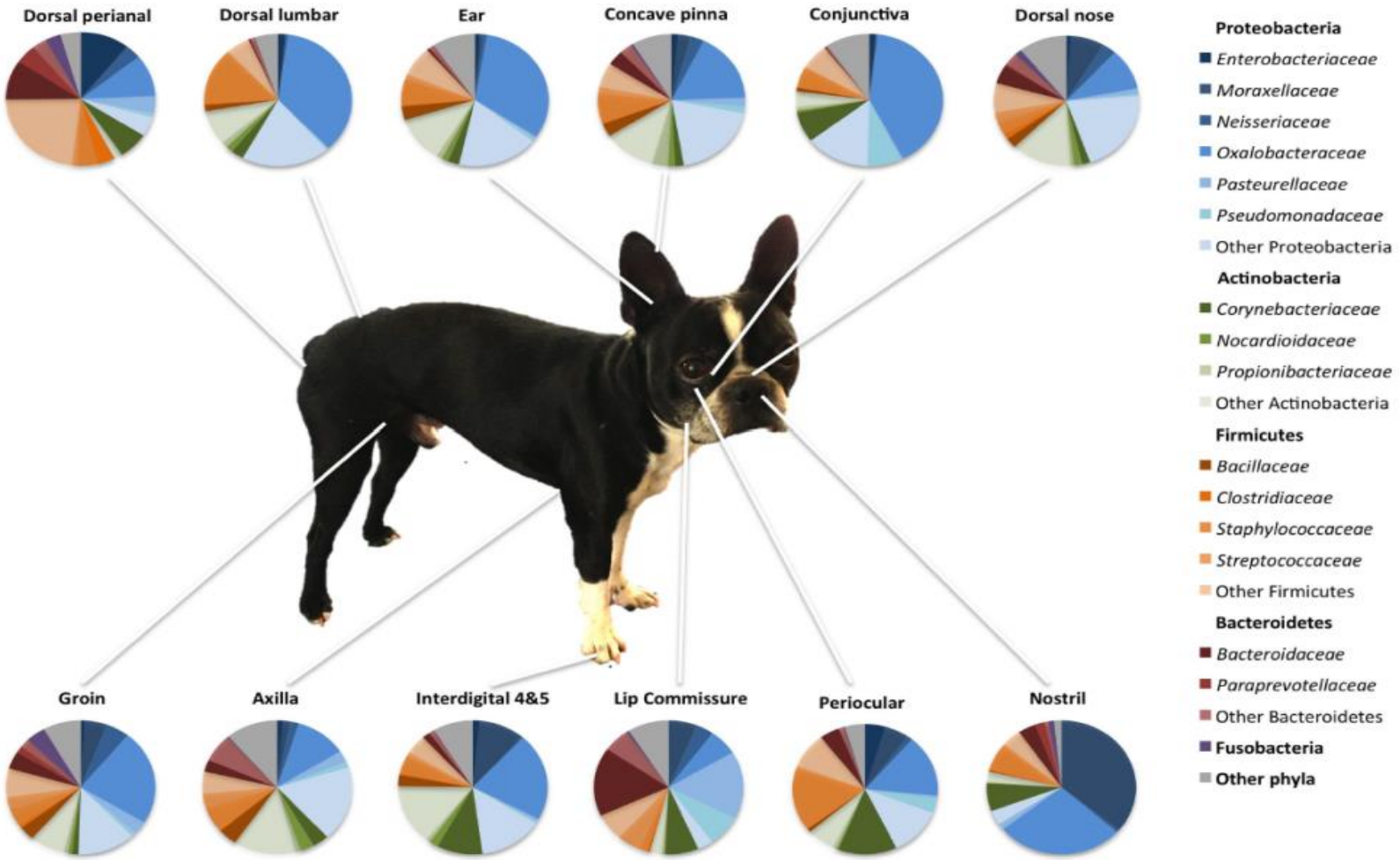


Figure 4. Bacterial phyla and families in healthy dogs. Average of most common bacterial phyla and families identified in different sites in the skin of healthy dogs.
doi:10.1371/journal.pone.0083197.g004

Hoffman et al., 2014

Diversidade da Microbiota

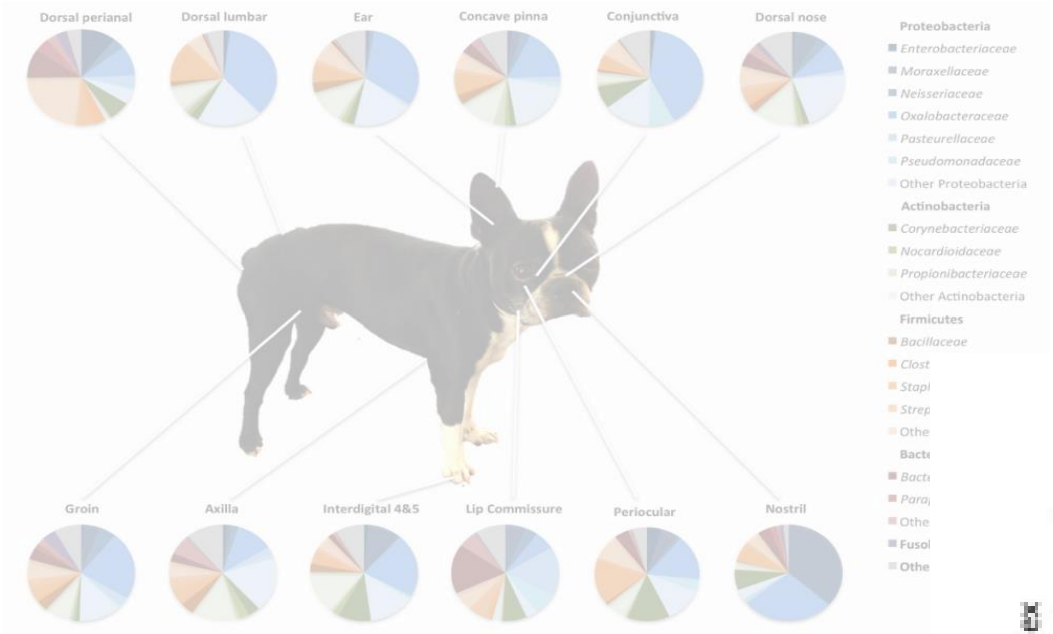
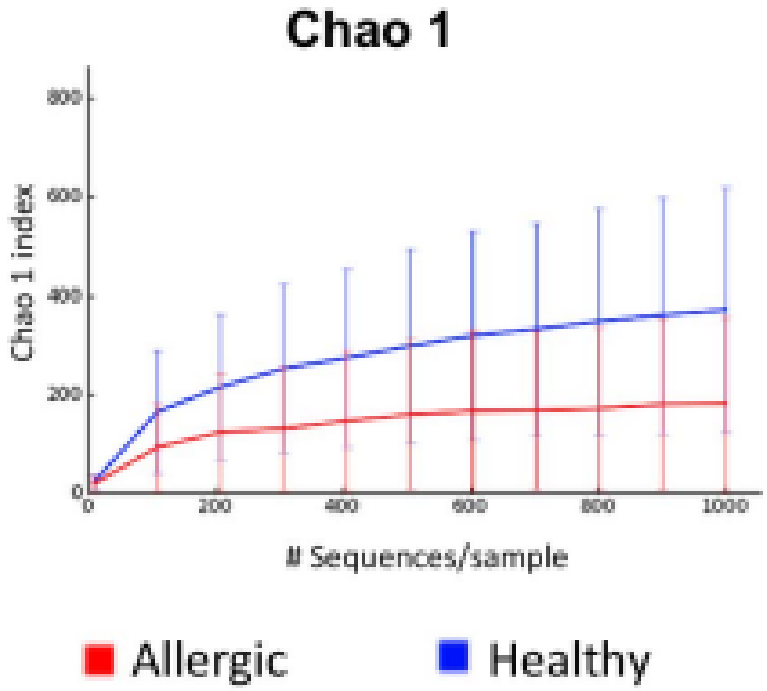
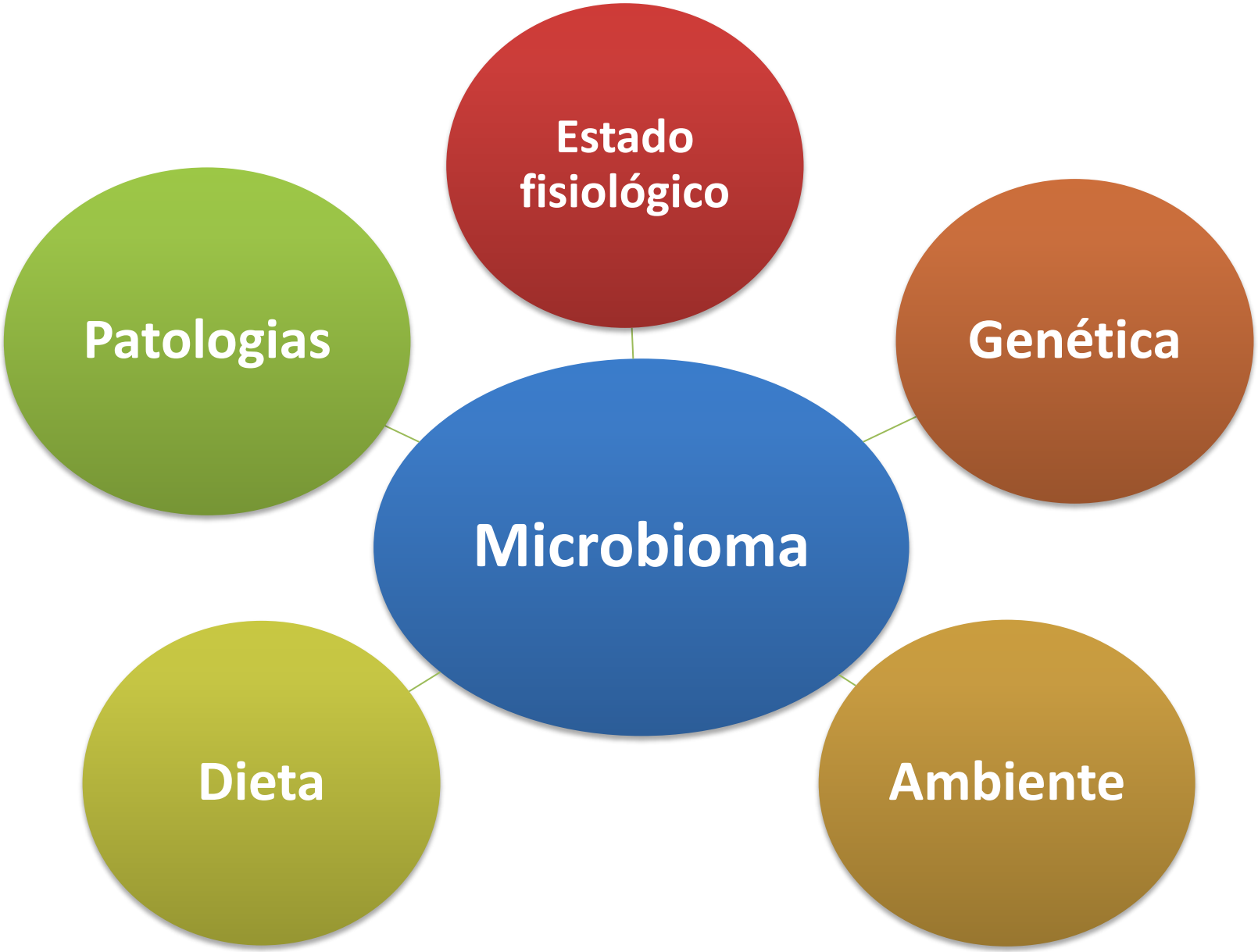


Figure 4. Bacterial phyla and families in healthy dogs. Average of most common bacterial phyla and families identified in differ
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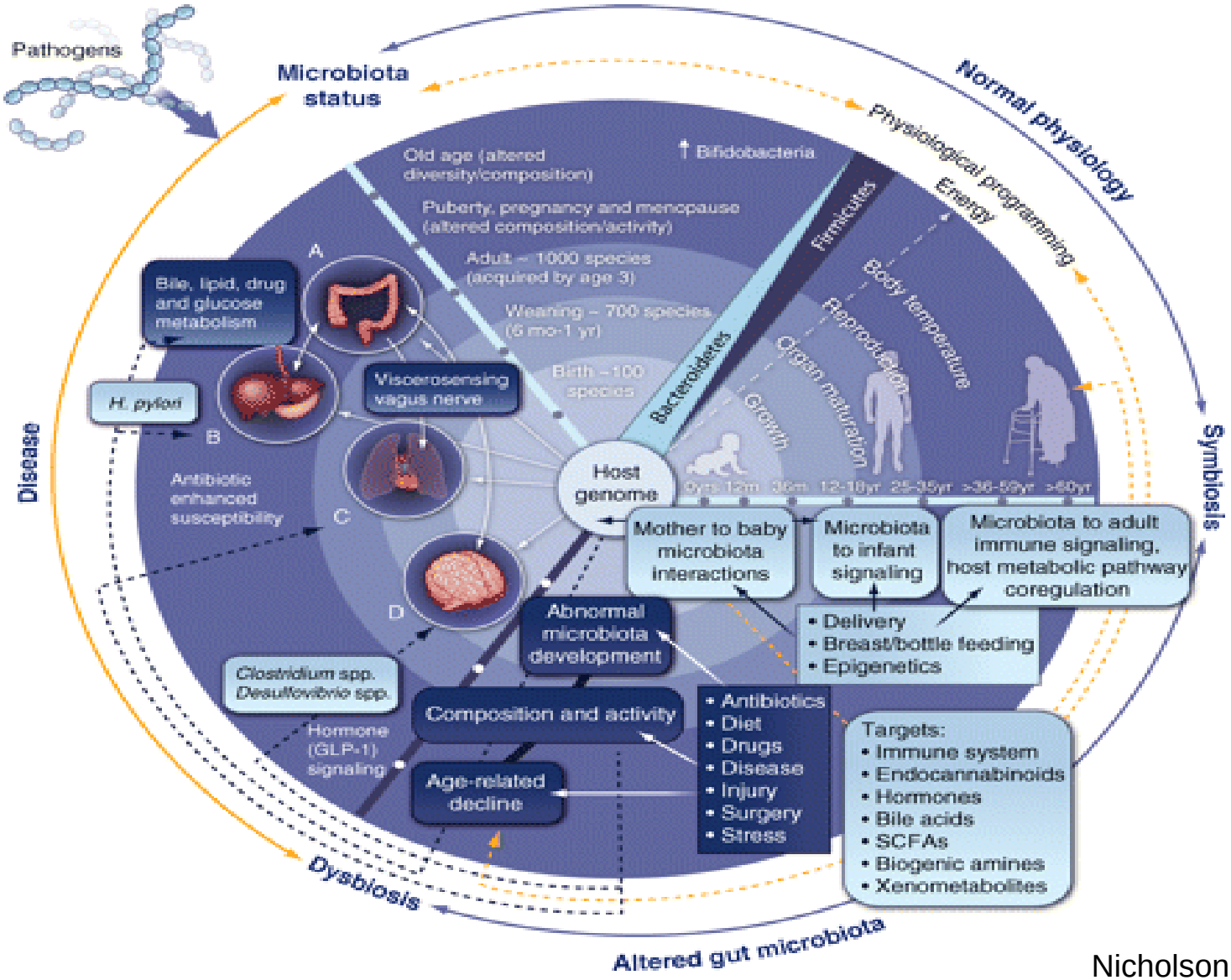


Hoffman et al., 2014

Modulação do Microbioma

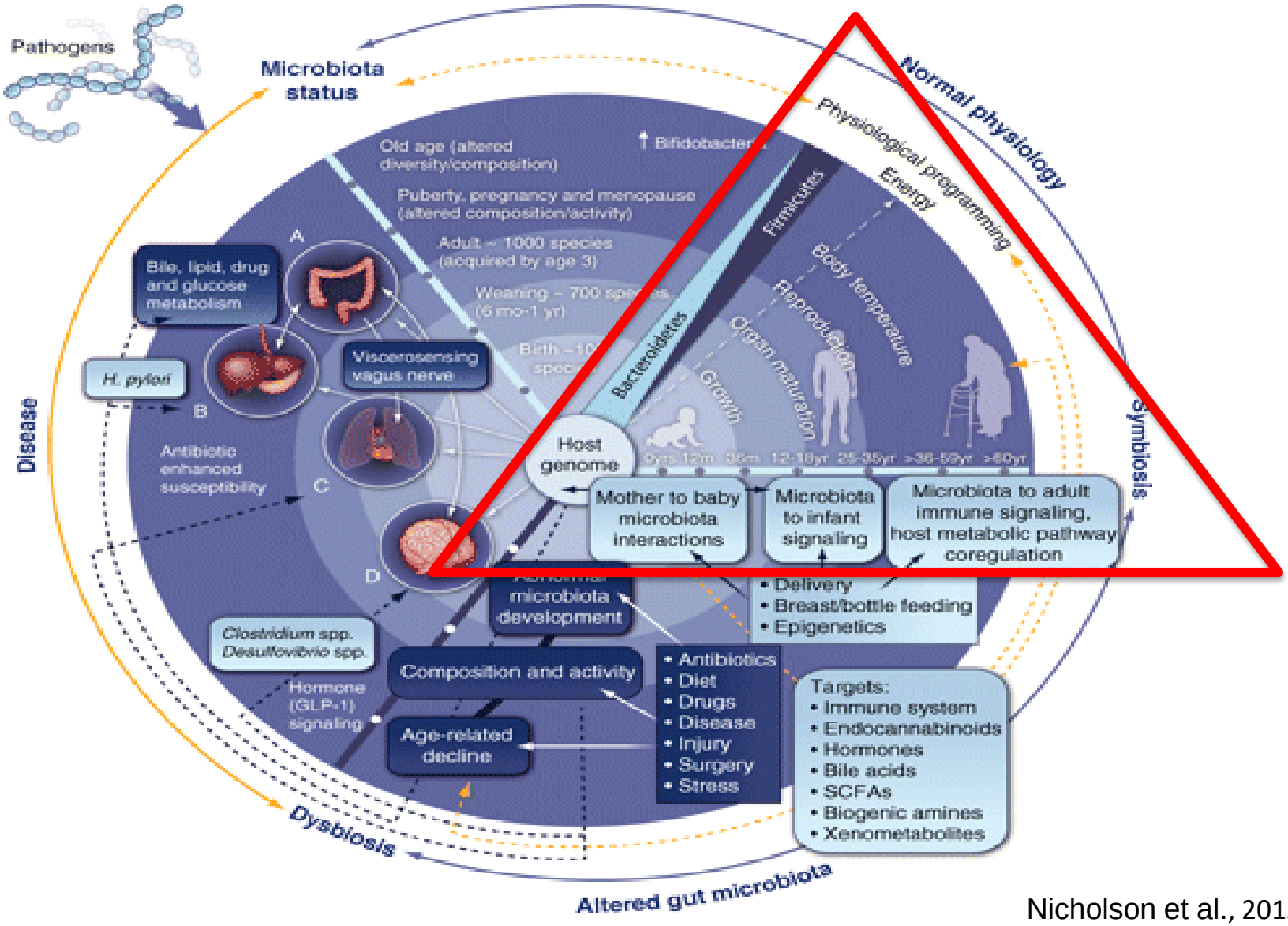


Relação Microbioma-Hospedeiro



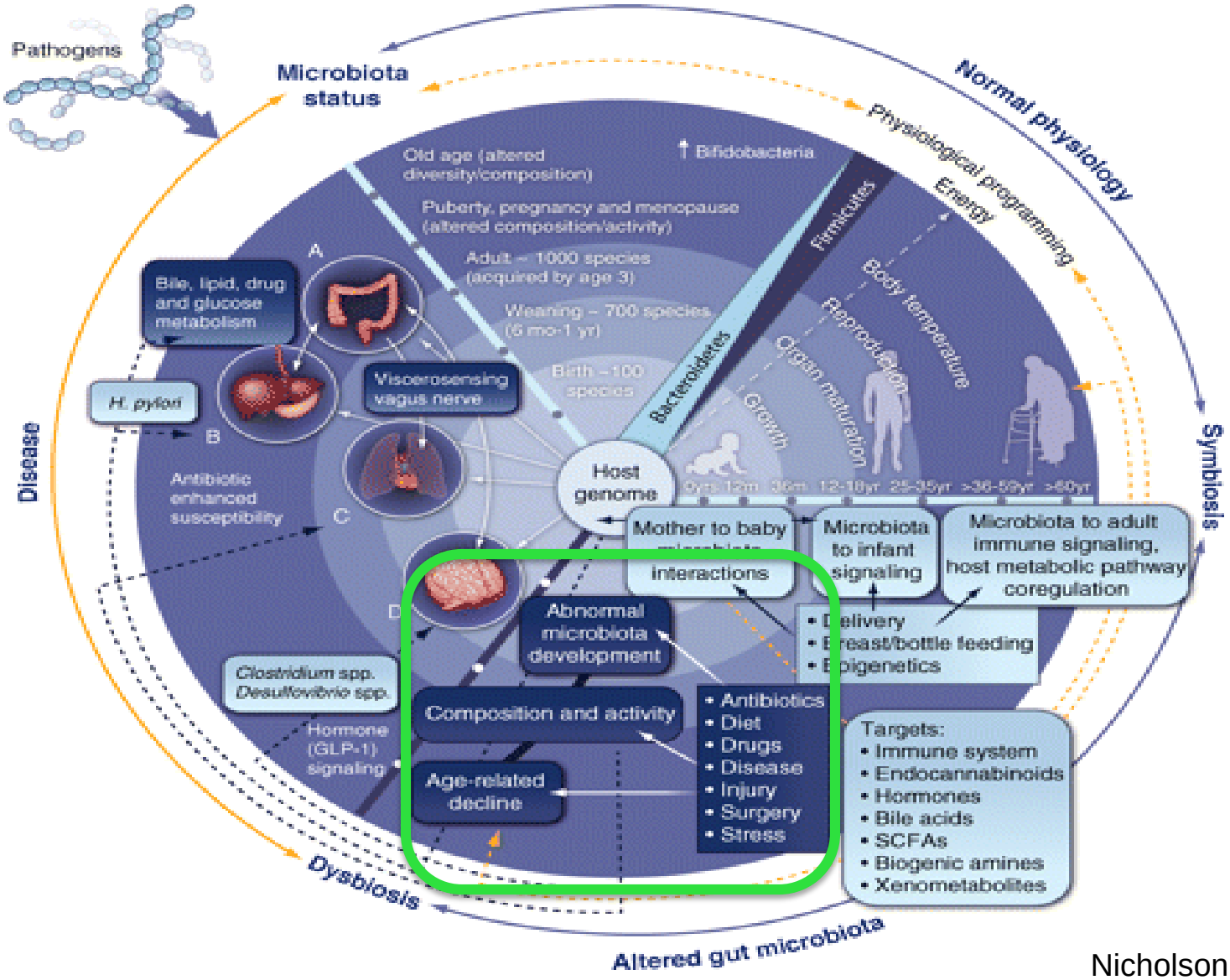
Nicholson et al., 2012

Relação Microbioma-Hospedeiro



Nicholson et al., 2012

Relação Microbioma-Hospedeiro



Nicholson et al., 2012

Relação Microbioma- Hospedeiro

Pontos chave

- Característica da comunidade microbiana?
  - Bactéria, arquea, fungos, vírus, etc.
- Existência de um núcleo microbiano indicativo de saúde or patologias?
  - Fatores que influenciam?

- Capacidade funcional do microbioma?
  - Que comunidades microbianas relevantes?
    - Função e metabolismo do hospedeiro?

Relação Microbioma- Hospedeiro

- Trato gastrointestinal é a interface mais importante entre a microbiota e o hospedeiro

- Ecosistema diverso e complexo
  - 500 – 1.000 diferentes espécies
  - Atividade simbiótica e/ ou de competição direta entre comunidades microbianas

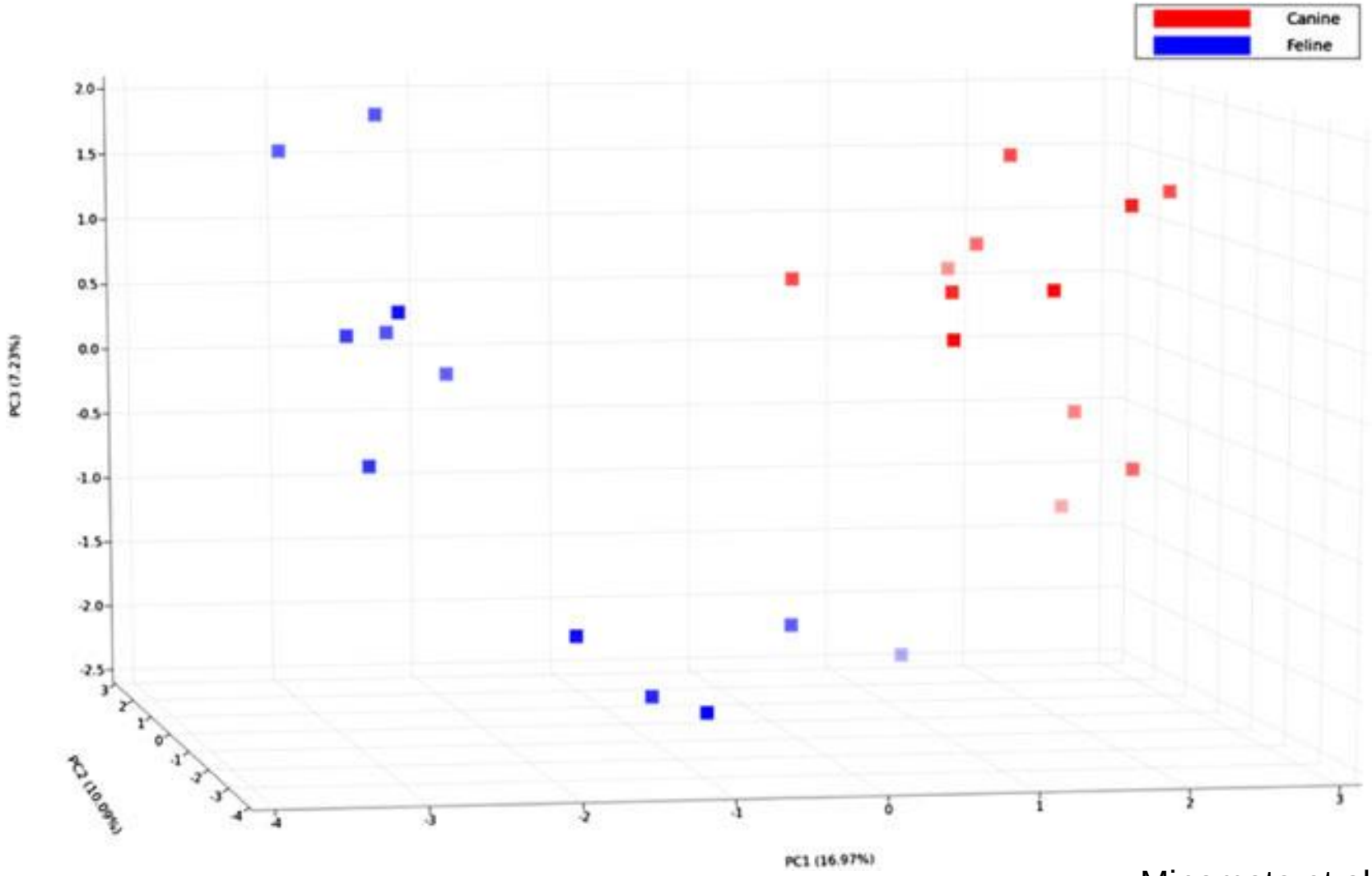
Microbiota: Trato Gastrointestinal Cães e Gatos

- Em cães adultos, densidade microbiana varia ao longo do TGI
  - Estômago: 10^4 to 10^5 unidades formadoras de colônia (CFU)/g;
    - Proteobacteria (>99%): *Helicobacter* spp.
    - Baixo pH, desfavorável para o crescimento e proliferação microbiana
  - Intestino delgado: 10^5 to 10^7 CFU/g
  - Colon: 10^9 to 10^{11} CFU/g



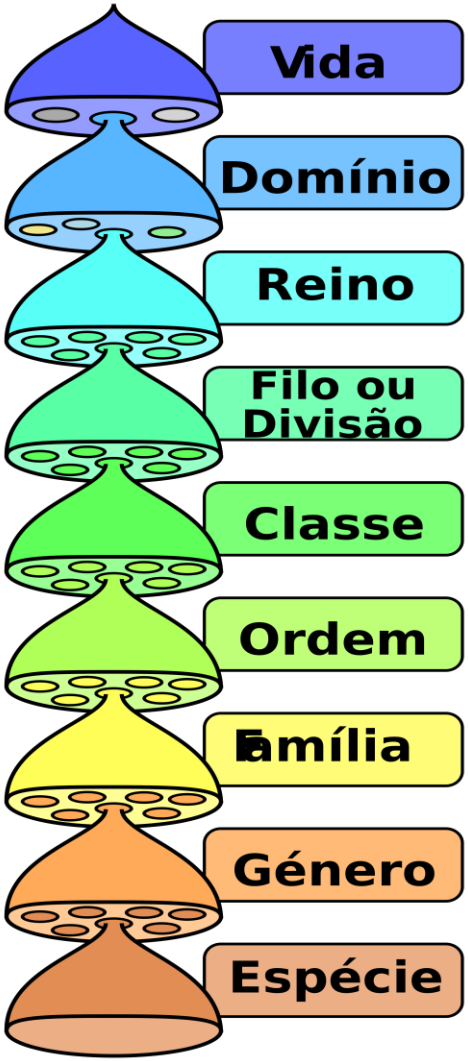
Benno et al., 1992; Johnston, 1999; Mentula et al., 2005; Garcia-Mazcorro et al., 2012; Hooda et al., 2012

PCoA - Comunidade Microbiana do TGI de Cães e Gatos



Minamoto et al., 2012

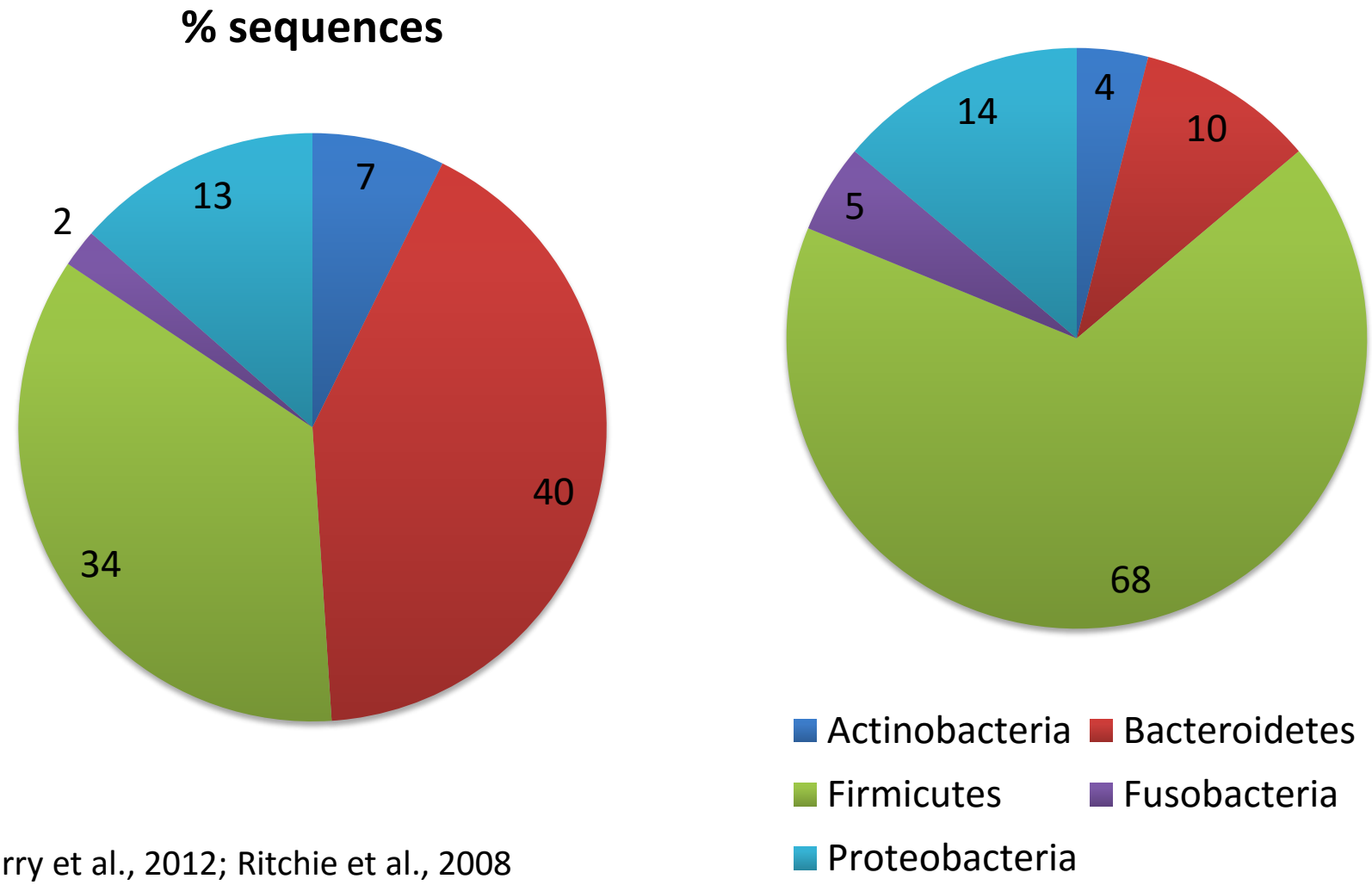
Composição Fecal Microbiana em Cães e Gatos: Filo



- Firmicutes
- Bacteroidetes
- Fusobacteria
- Proteobacteria
- Actinobacteria

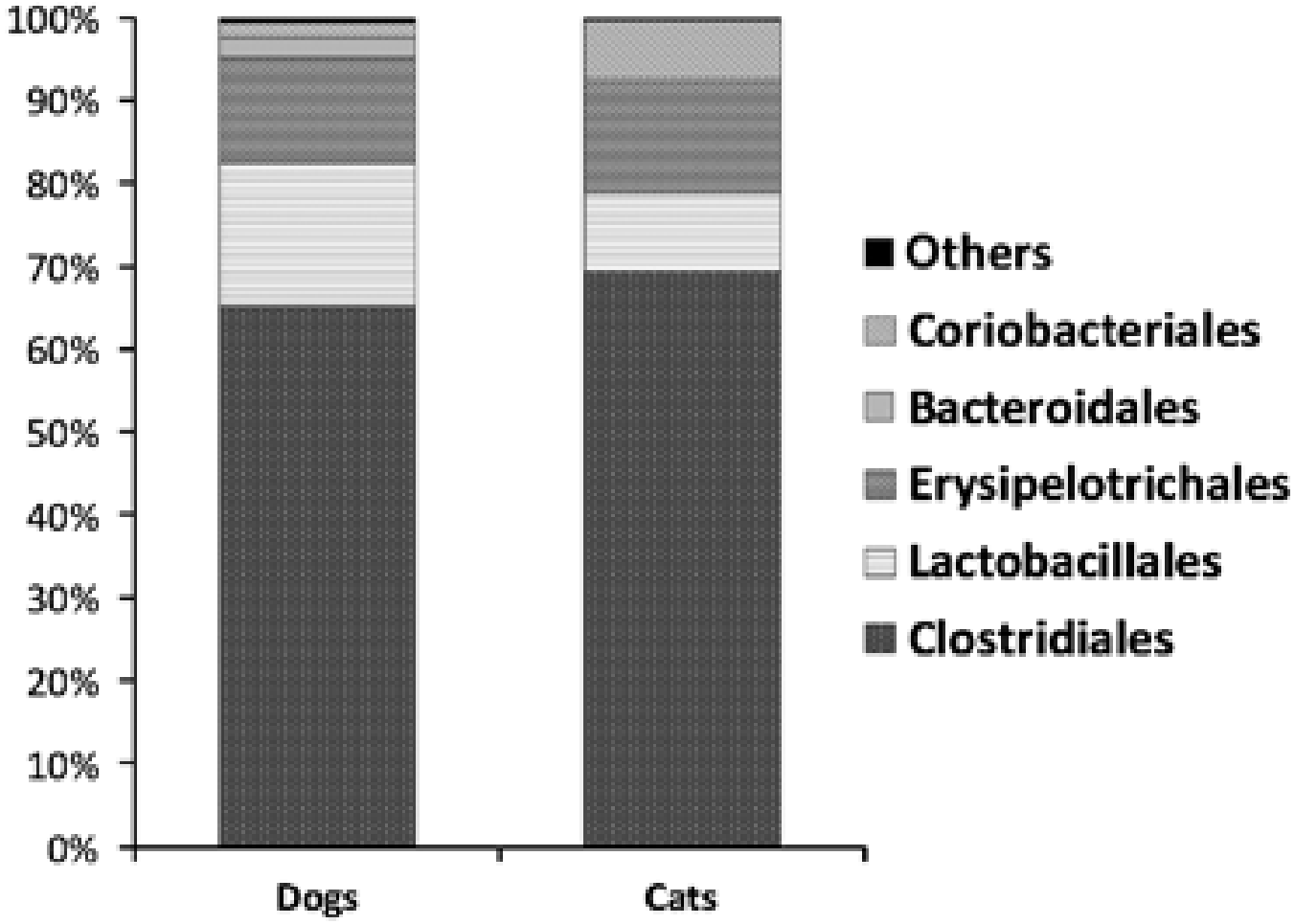
Hooda et al., 2012; Minamoto et al., 2012

Filos Predominantes na Microbiota Fecal de Felinos



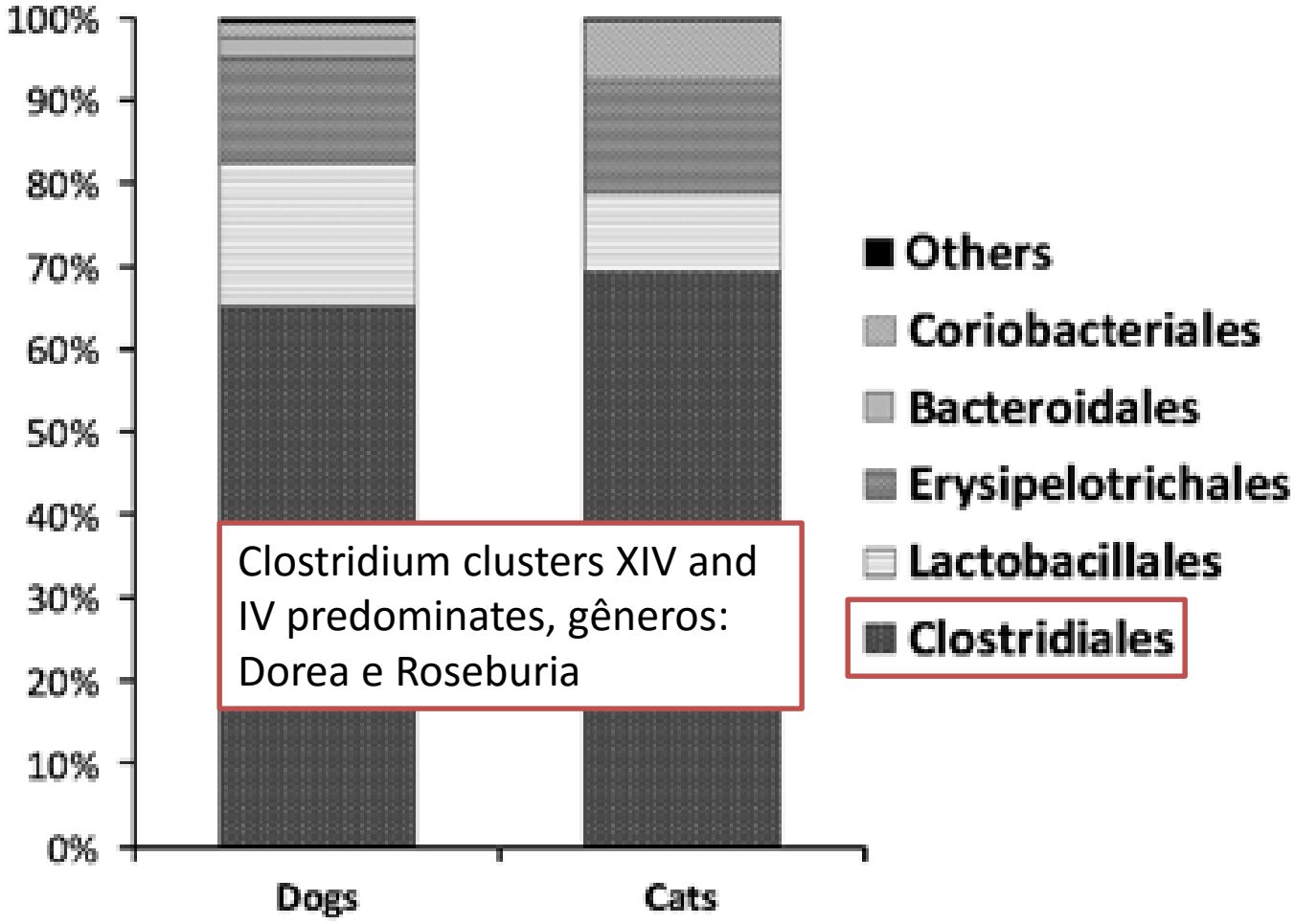
Barry et al., 2012; Ritchie et al., 2008

Composição Fecal Microbiana em Cães e Gatos: Ordem



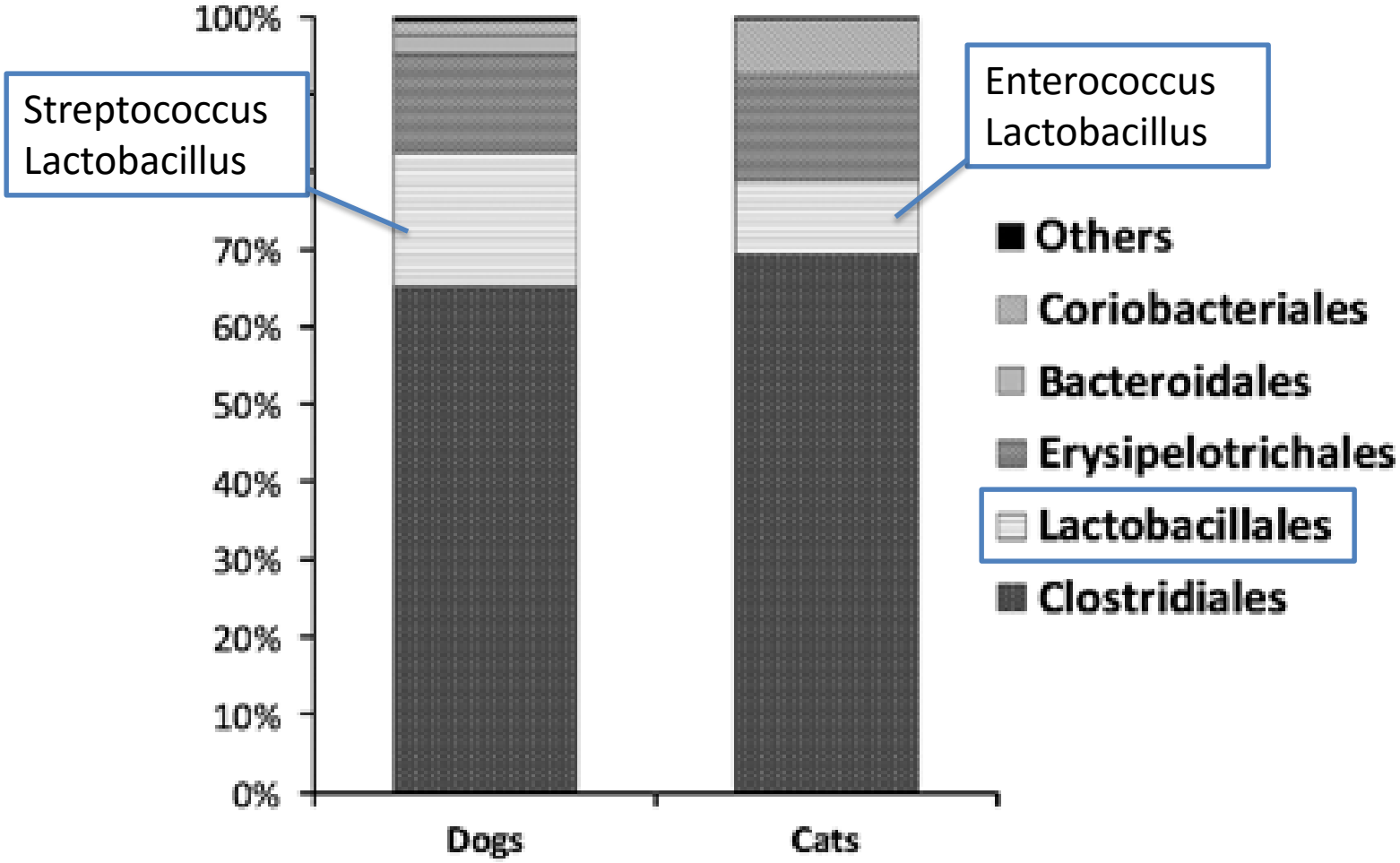
Handl et al., 2011

Composição Fecal Microbiana em Cães e Gatos: Ordem



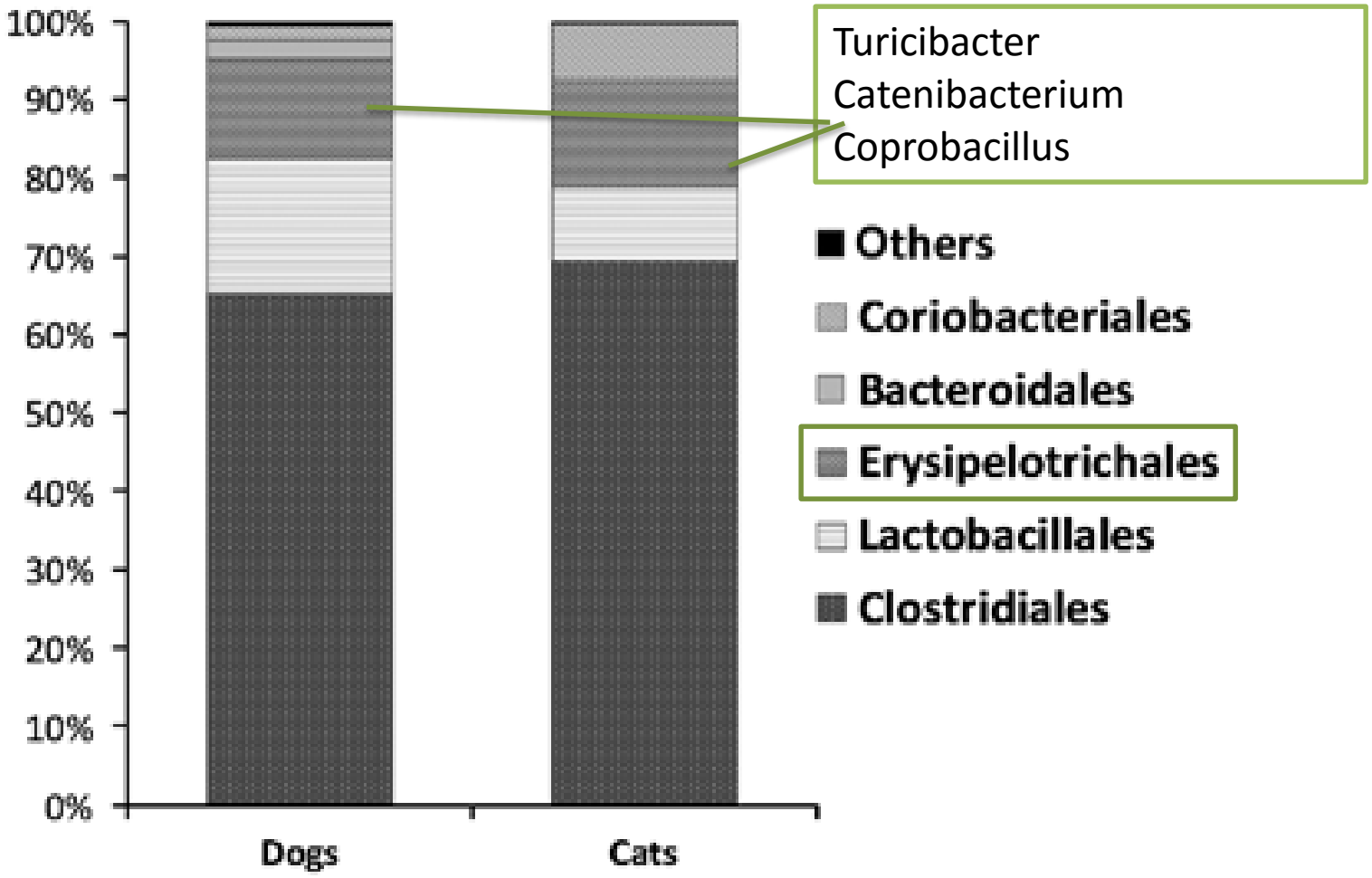
Handl et al., 2011

Composição Fecal Microbiana em Cães e Gatos: Ordem



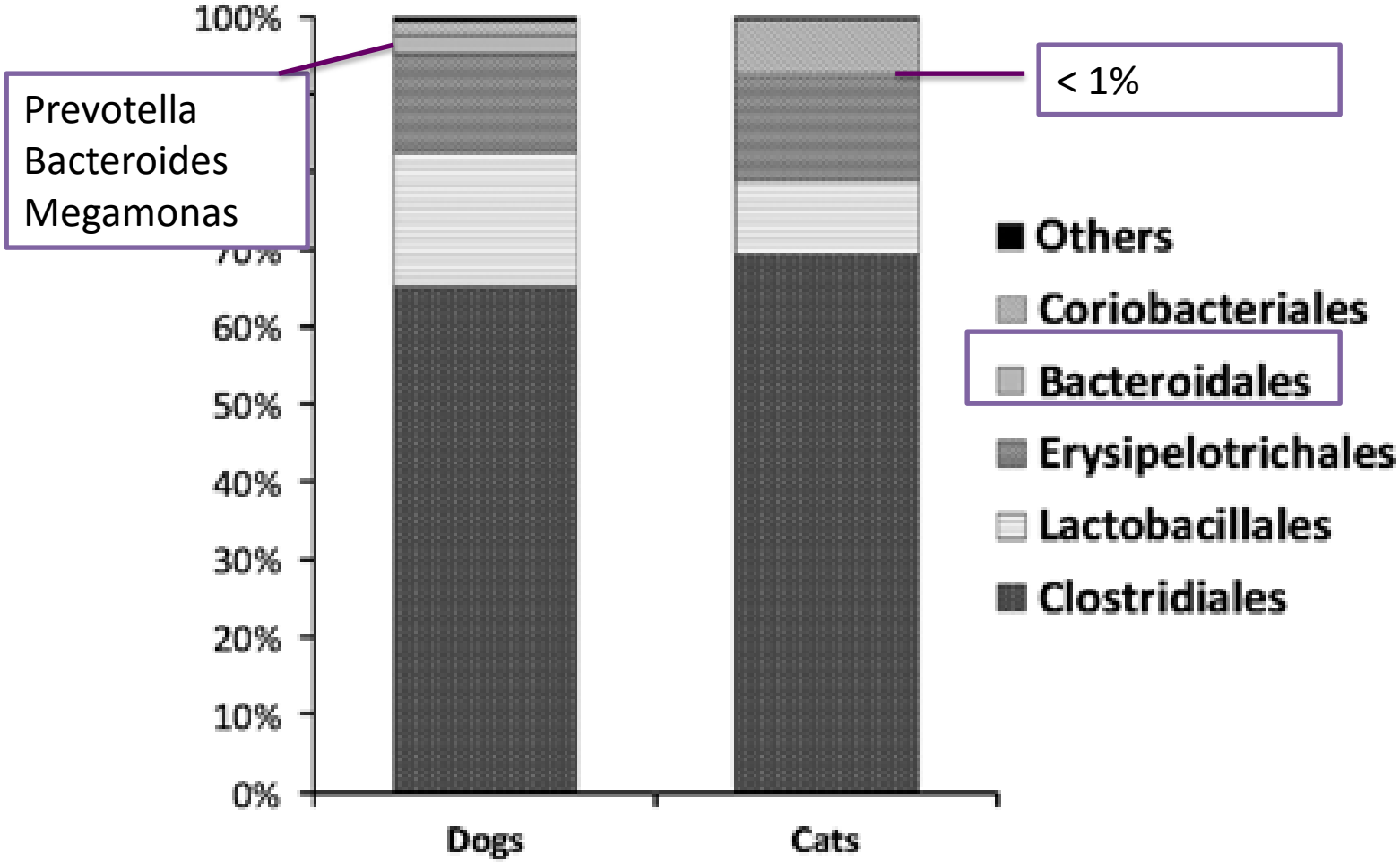
Handl et al., 2011

Composição Fecal Microbiana em Cães e Gatos: Ordem



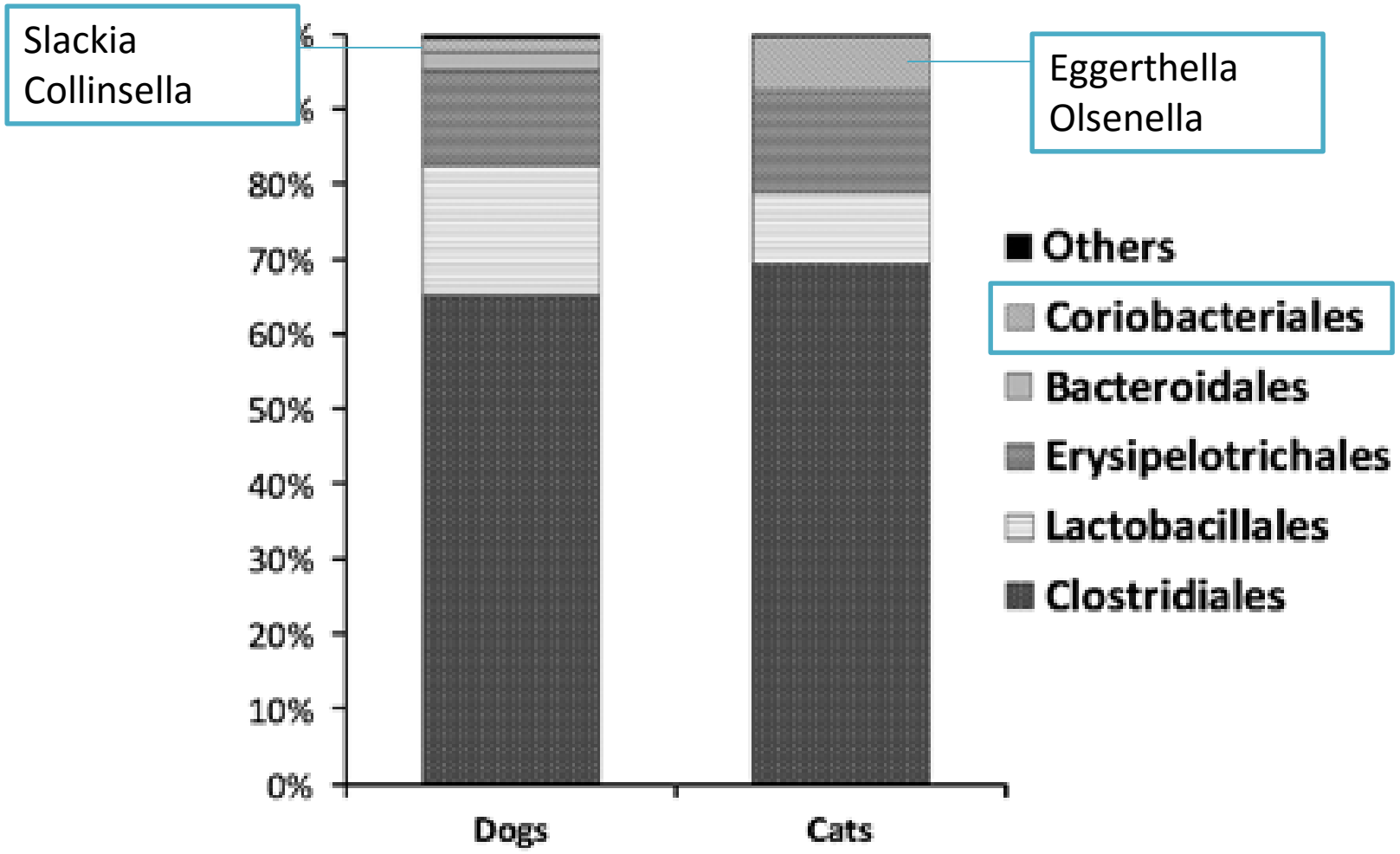
Handl et al., 2011

Composição Fecal Microbiana em Cães e Gatos: Ordem



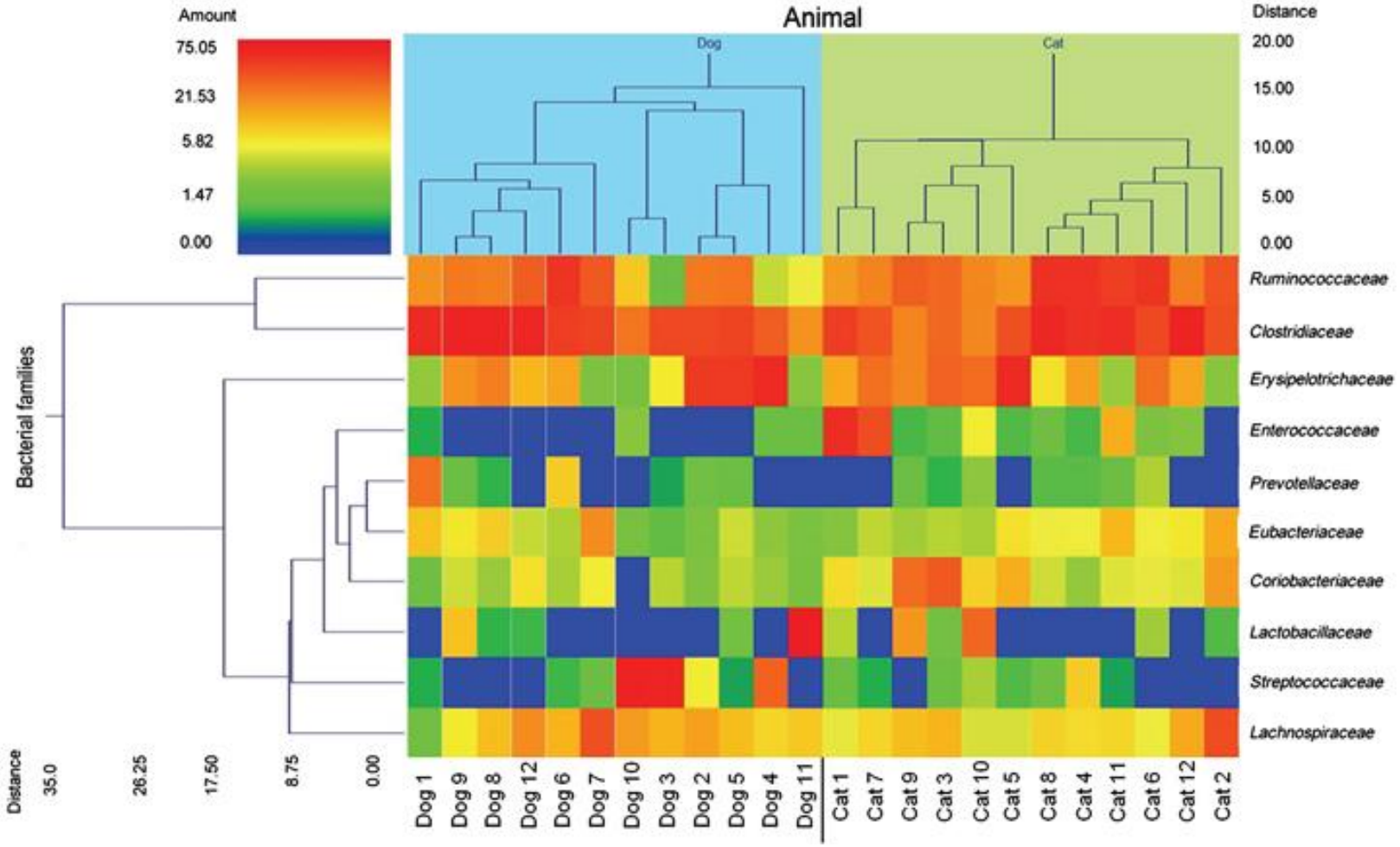
Handl et al., 2011

Composição Fecal Microbiana em Cães e Gatos: Ordem



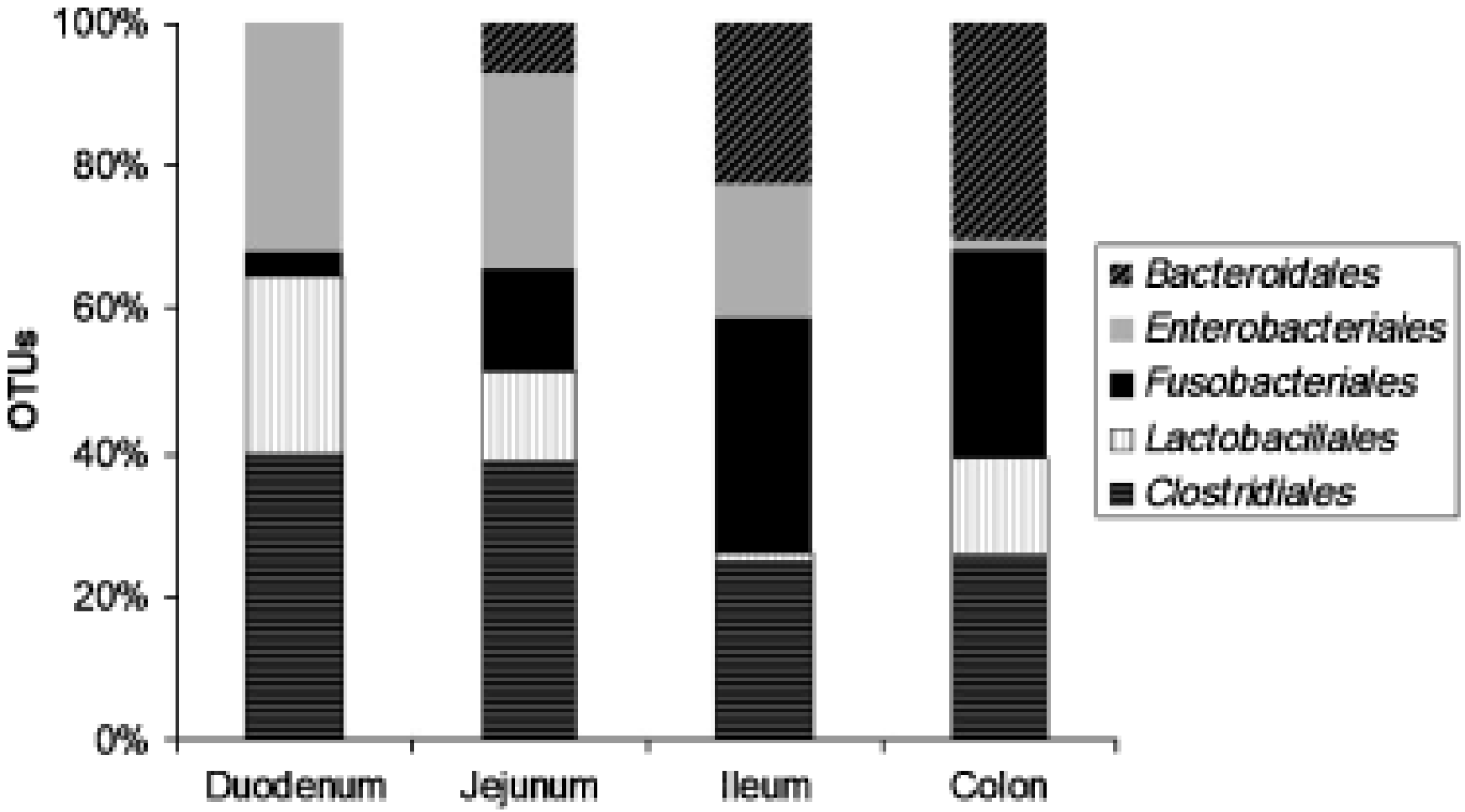
Handl et al., 2011

Dendrograma Principais Familias Bacterianas Fecais em Cães e Gatos Adultos



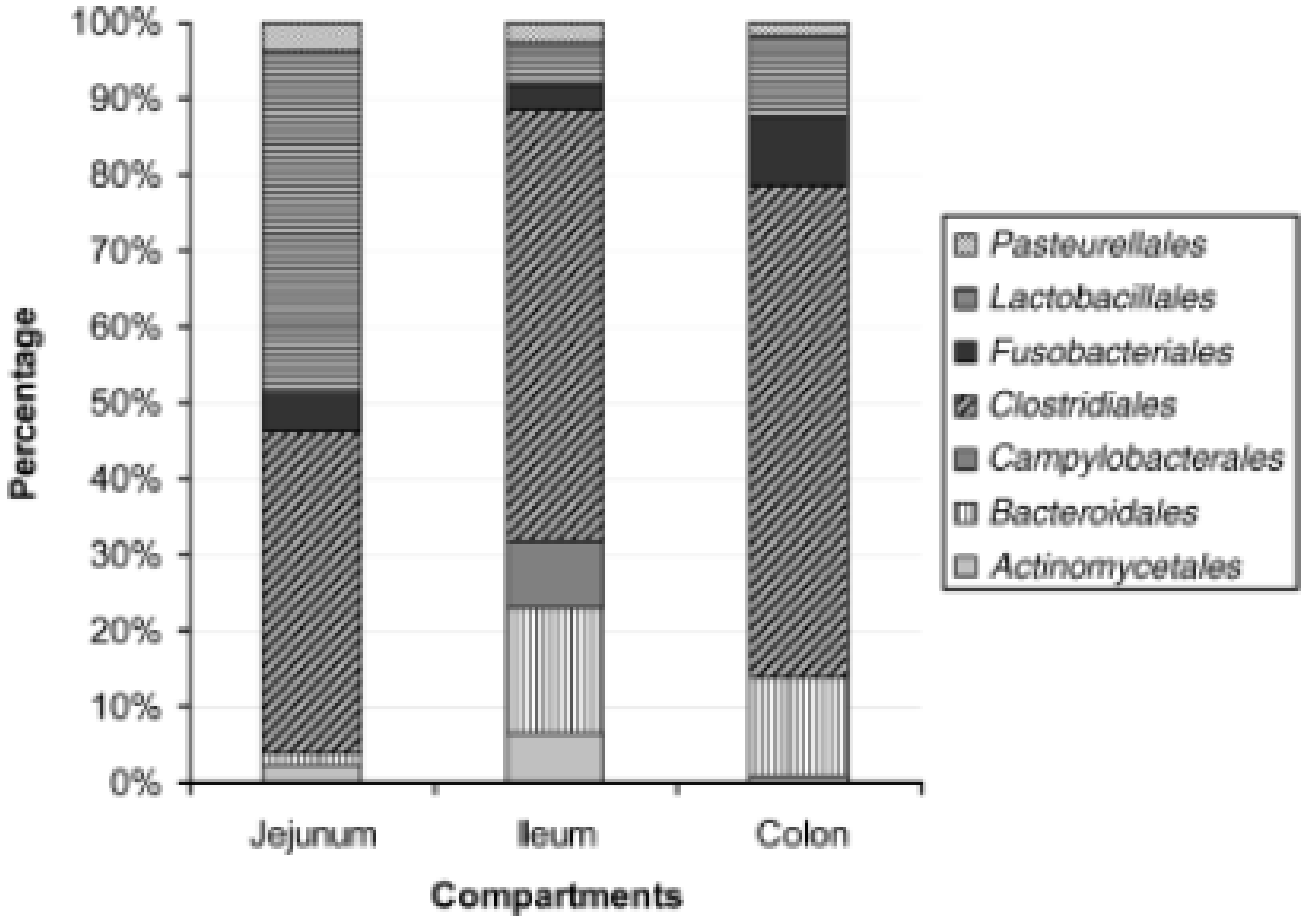
Handl et al., 2011

Diversidade da Microbiota
TGI Canino: Ordens



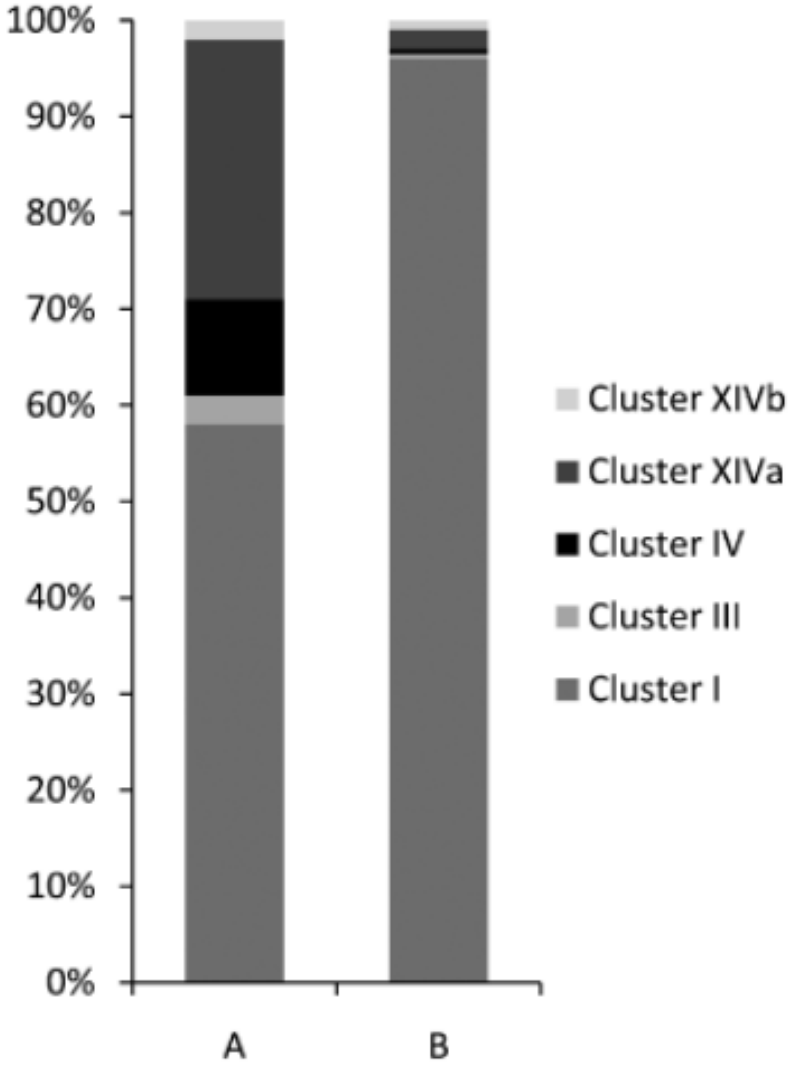
Suchodolsky et al., 2008

Diversidade da Microbiota
TGI Felino: Ordens



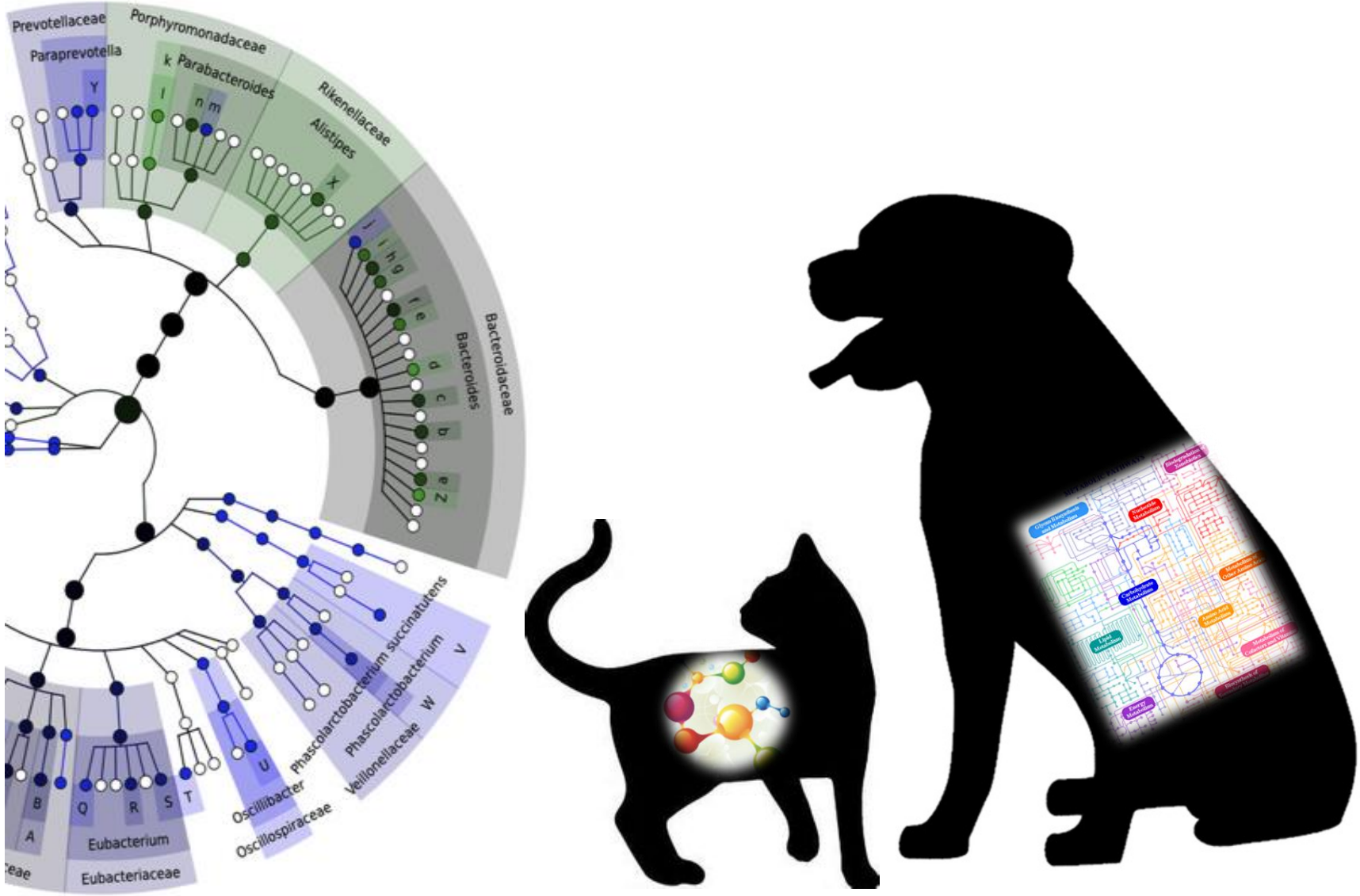
Ritchie et al., 2008

Diversidade da Microbiota
Gatos saudáveis Convencionais (A) e SPF (B)

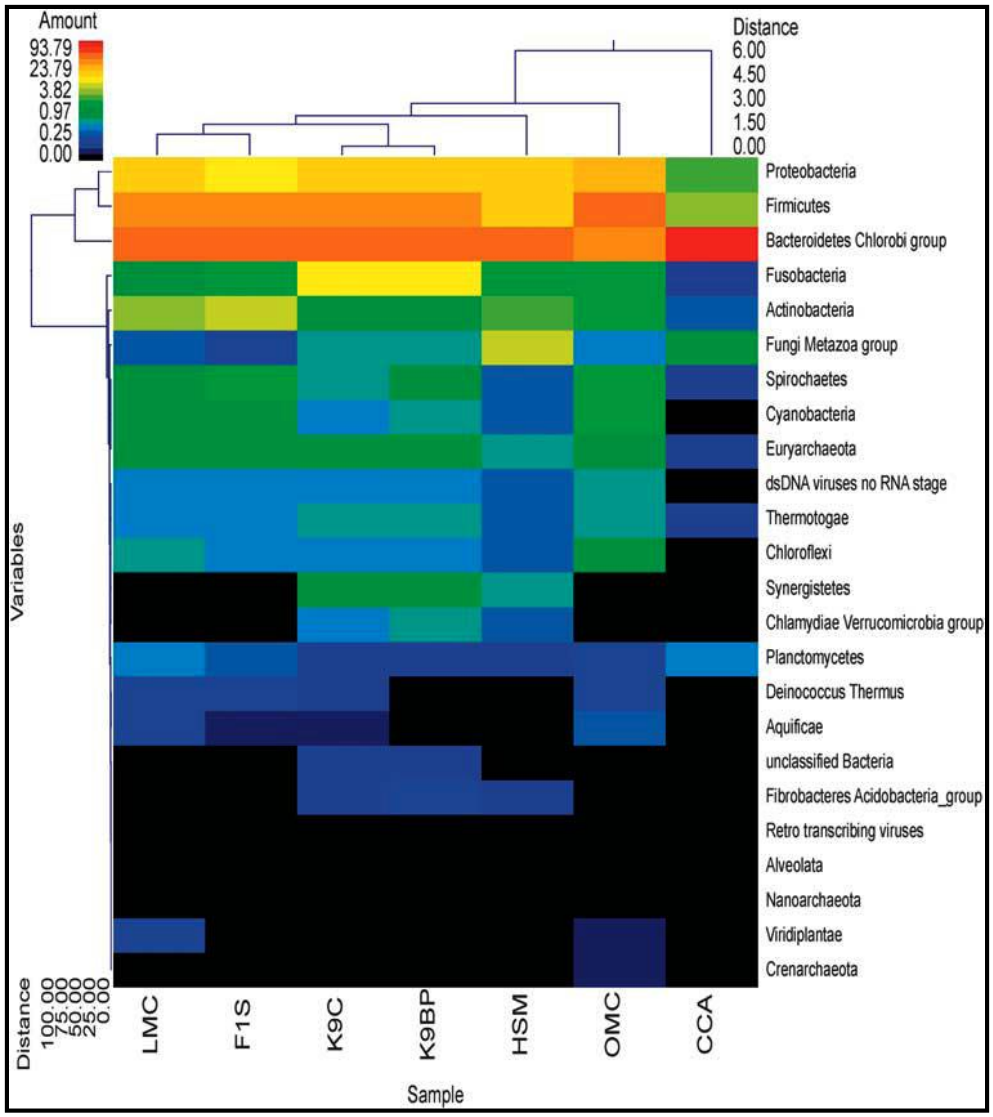


Ritchie et al., 2008

Filogenia .. Apenas o Primeiro Passo



Microbioma Gastrointestinal Canino

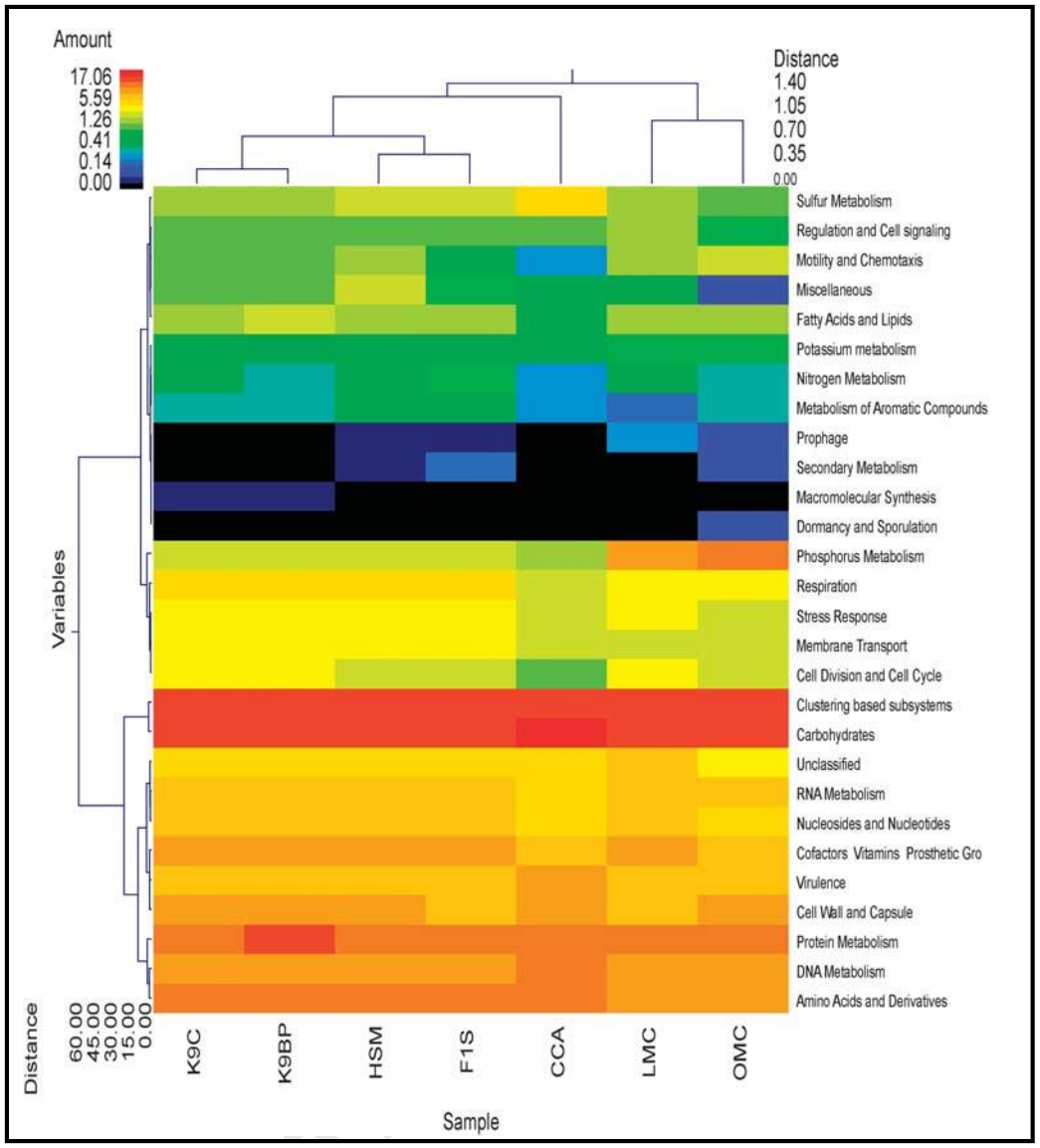


Principais filos:

- Proteobacteria (13-15%)
- Fimicutes (31-35%)
- Bacteriodetes (37%)
- Fusobacteria (7-8%)
- Actinobacteria (1%)

Swanson et al., 2011

Microbioma Fecal Canino

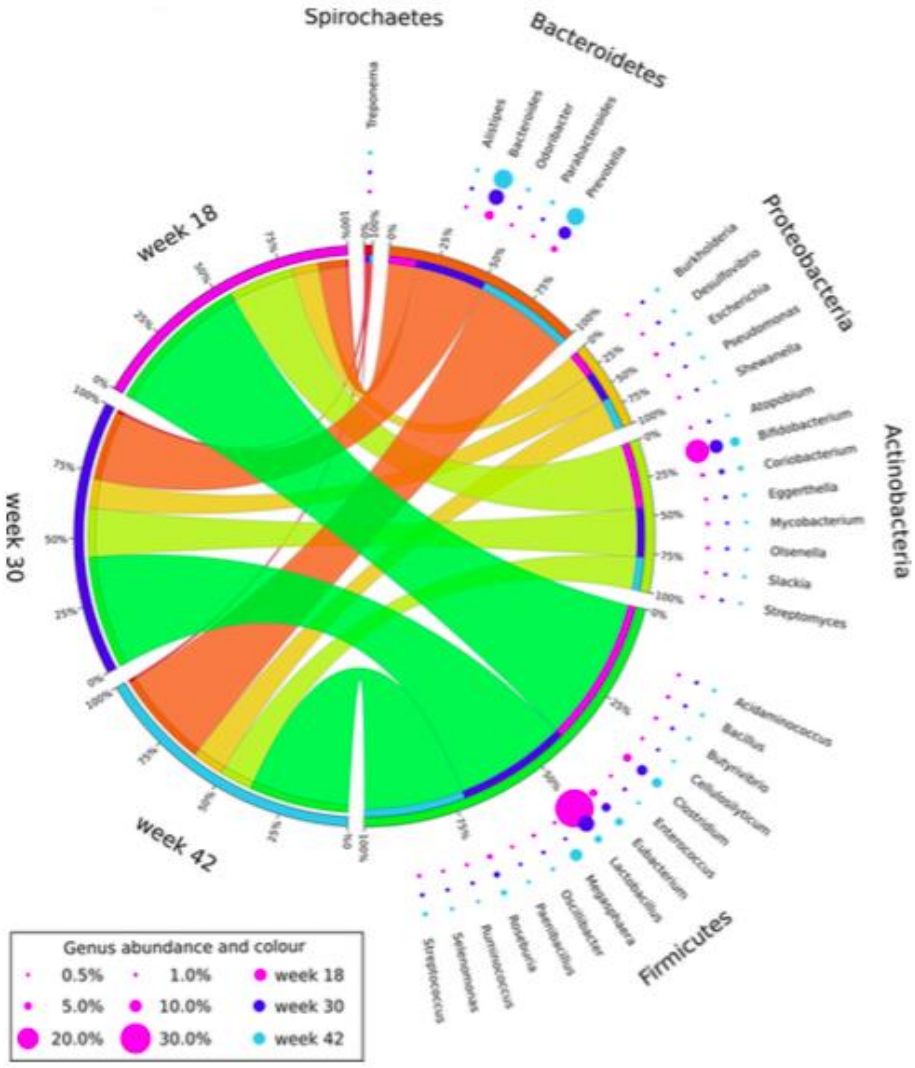


Principais categorias metabolicas:

- Carboidratos (~13%)
- Metab. proteínas (~ 9%)
- Metab. DNA(~ 7%)
- Biosíntese de parede cellular(~ 8%)
- Derivados de AA (~7%)
- Virulência (~ 7%)
- Vitaminas e co-fatores de metabolismo (~ 6%)

Swanson et al., 2011

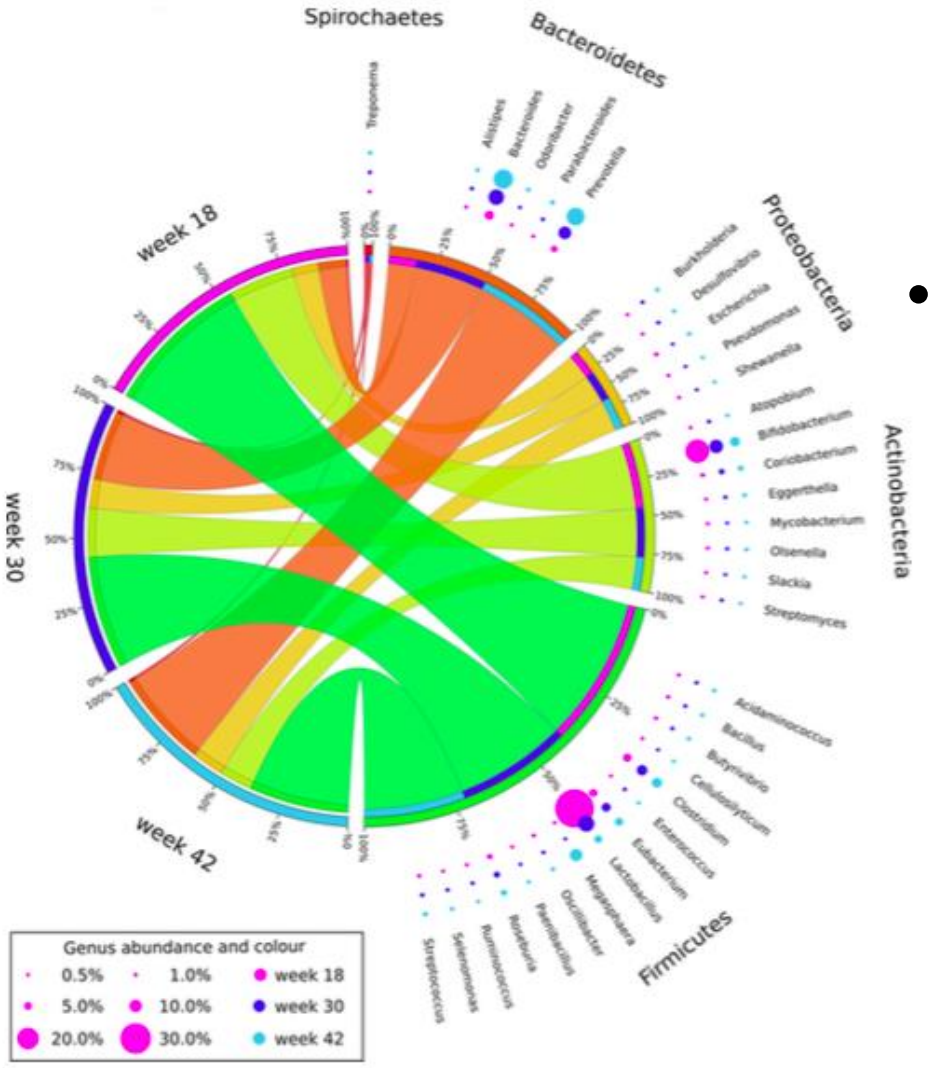
Microbioma Fecal de Felinos: Estudo Longitudinal Efeito da Castração



- 30 filhotes de gatos
  - 16 machos
  - 14 fêmeas
- Idade de castração
  - 19 semanas (precoce)
  - 31 semanas (tradicional)
- Coleta fecal
  - 18, 30 e 42semanas de idade
- Dieta: única extrusada

Deusch et al., 2015

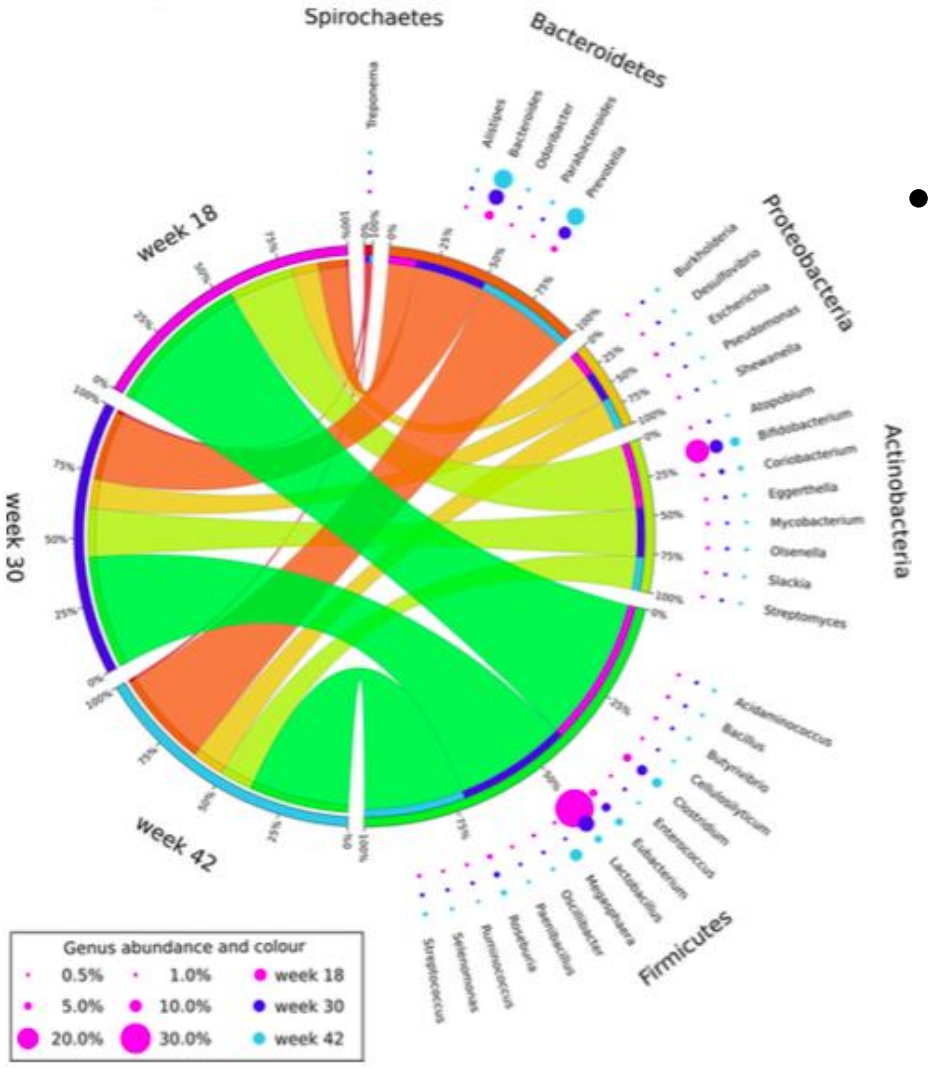
Microbioma Fecal de Felinos: Estudo Longitudinal Efeito da Castração



- 18 semanas
  - Lactobacillus (35%)
  - Bifidobactéria (20%)

Deusch et al., 2015

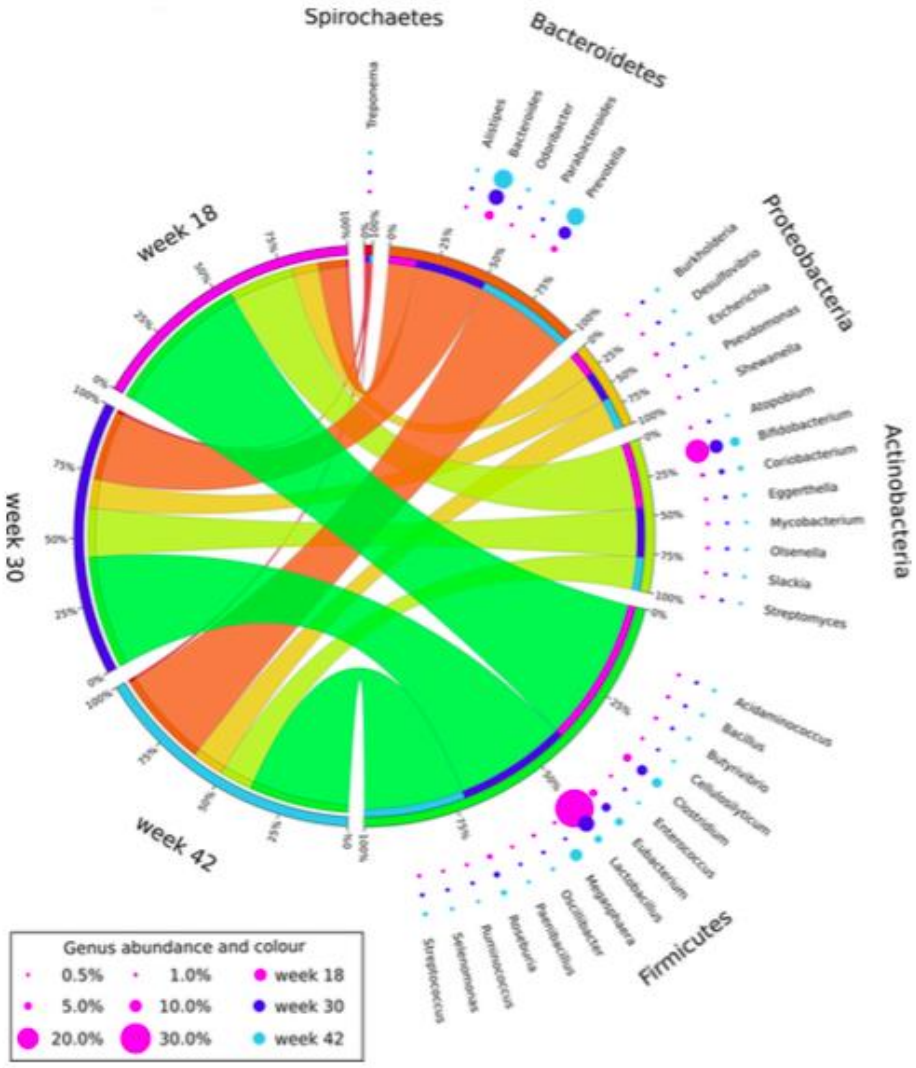
Microbioma Fecal de Felinos: Estudo Longitudinal



- 30 semanas
  - Maior diversidade
    - Bacteroides
    - Prevotella
    - Bifidobacteria
    - Clostridium
    - Lactobacillus

Deusch et al., 2015

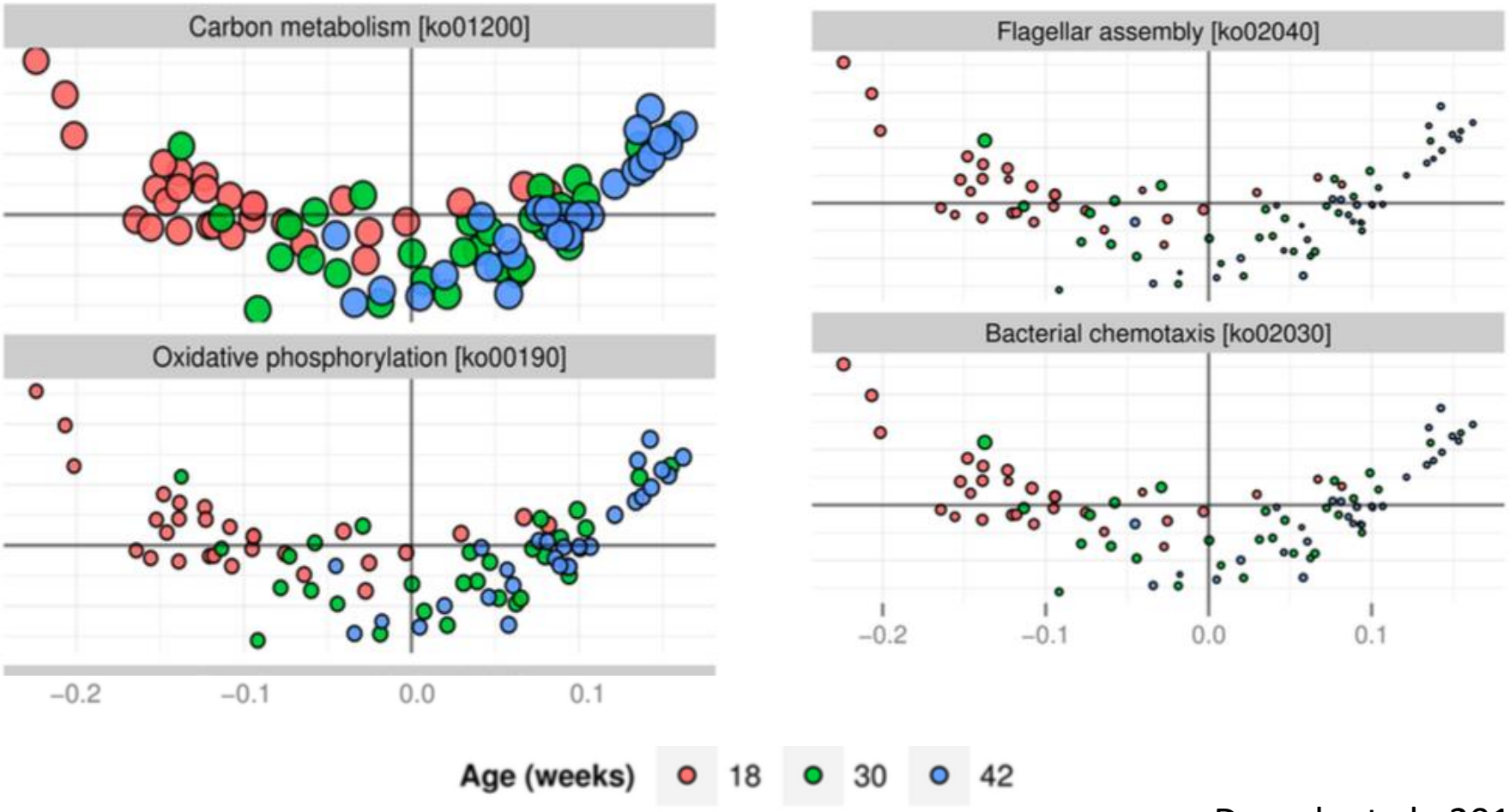
Microbioma Fecal de Felinos: Estudo Longitudinal



- 42 semanas
  - Estabilização da diversidade
  - Principais grupos taxonômicos
    - Bacteroides
    - Prevotella
    - Megasphaera (>8%)

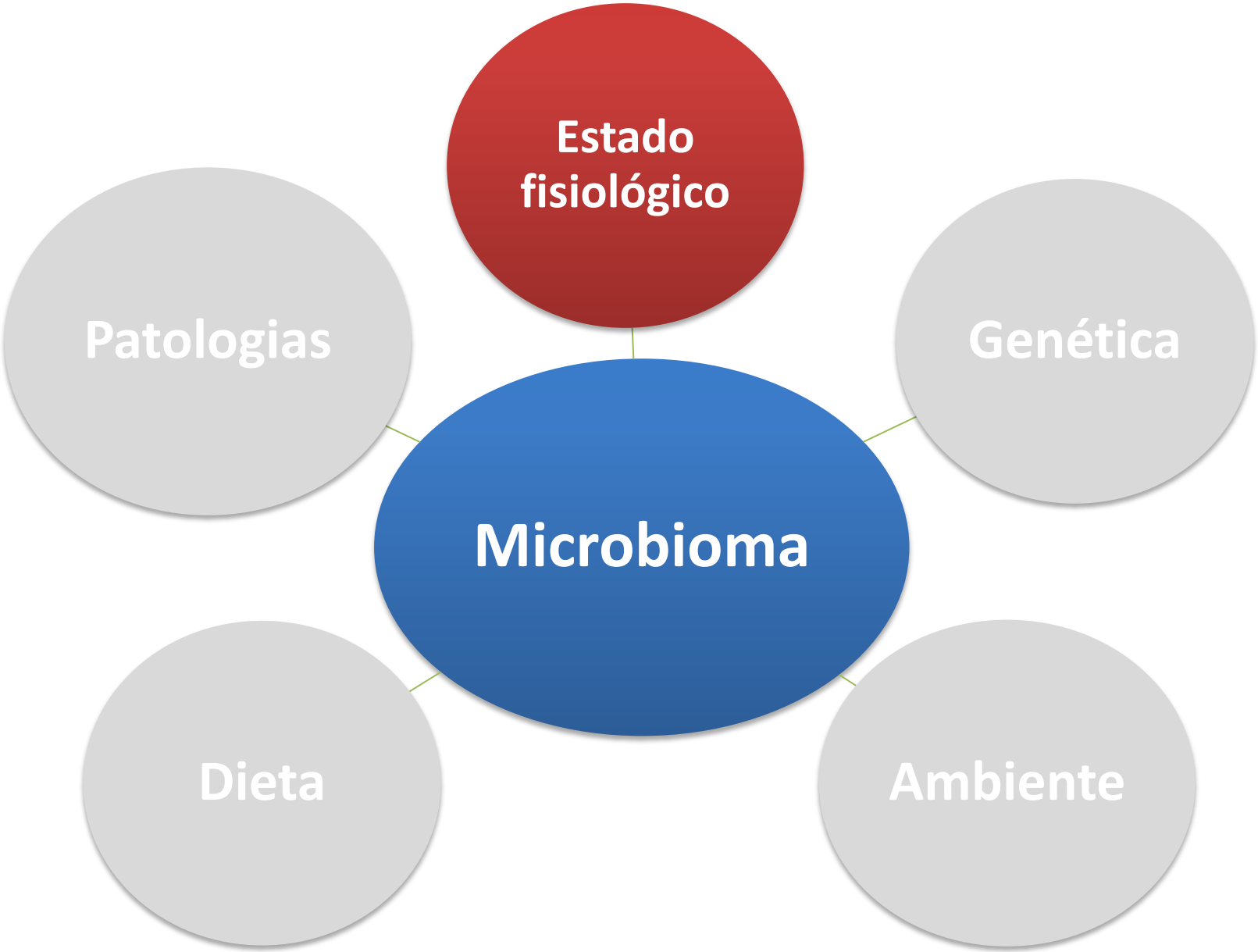
Deusch et al., 2015

Microbioma Fecal de Felinos: Estudo Longitudinal



Deusch et al., 2015

Alterações da Microbiota



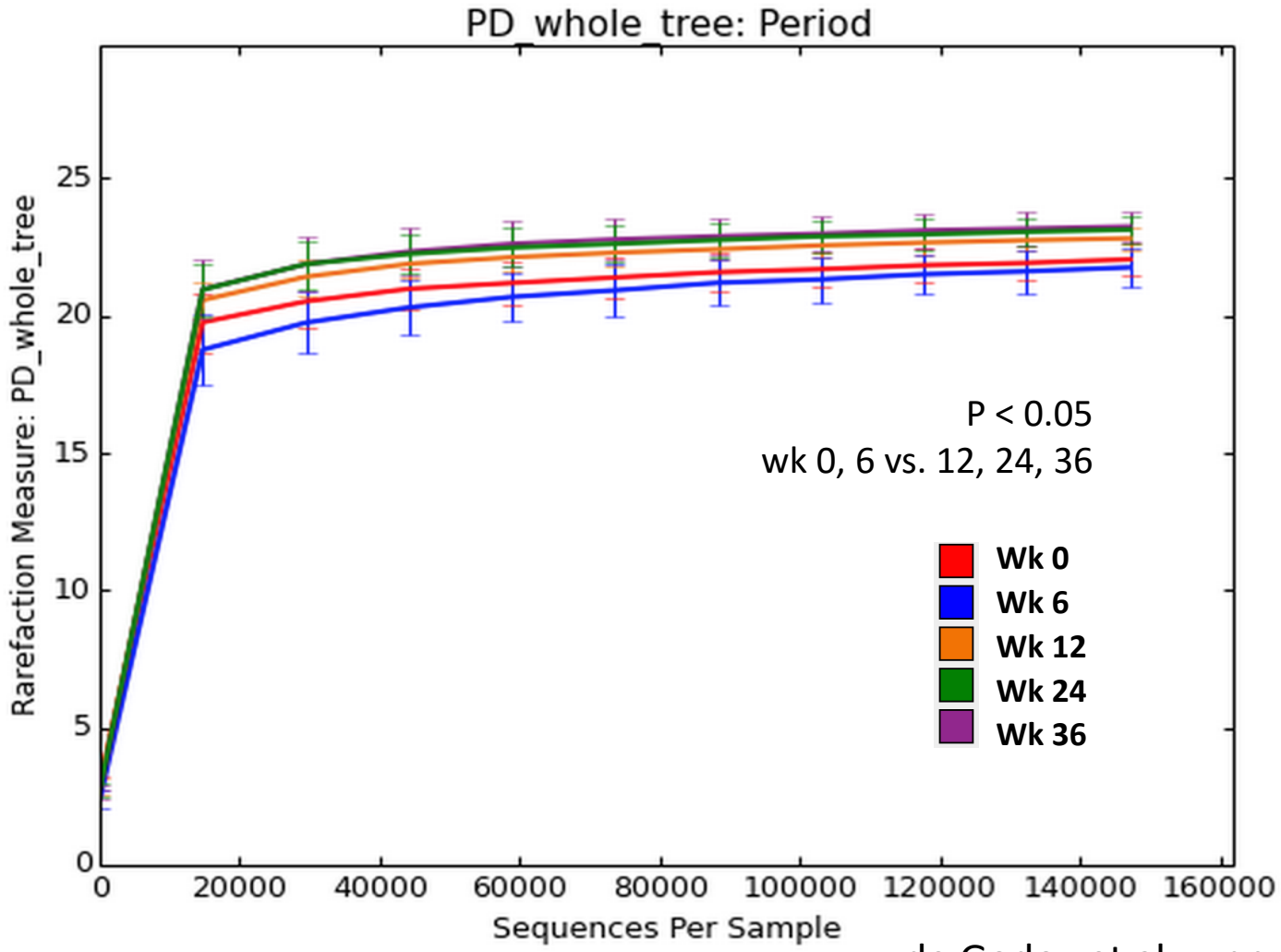
Relação Microbioma- Hospedeiro



- Gatos geriátricos – Taxas Predominantes
  - Actinobacteria
    - Coriobacteriaceae
    - Bifidobacterium
  - Firmicutes
    - Clostridium cluster XIV
    - Bactérias que produzem ácido lático
      - Lactobacillus

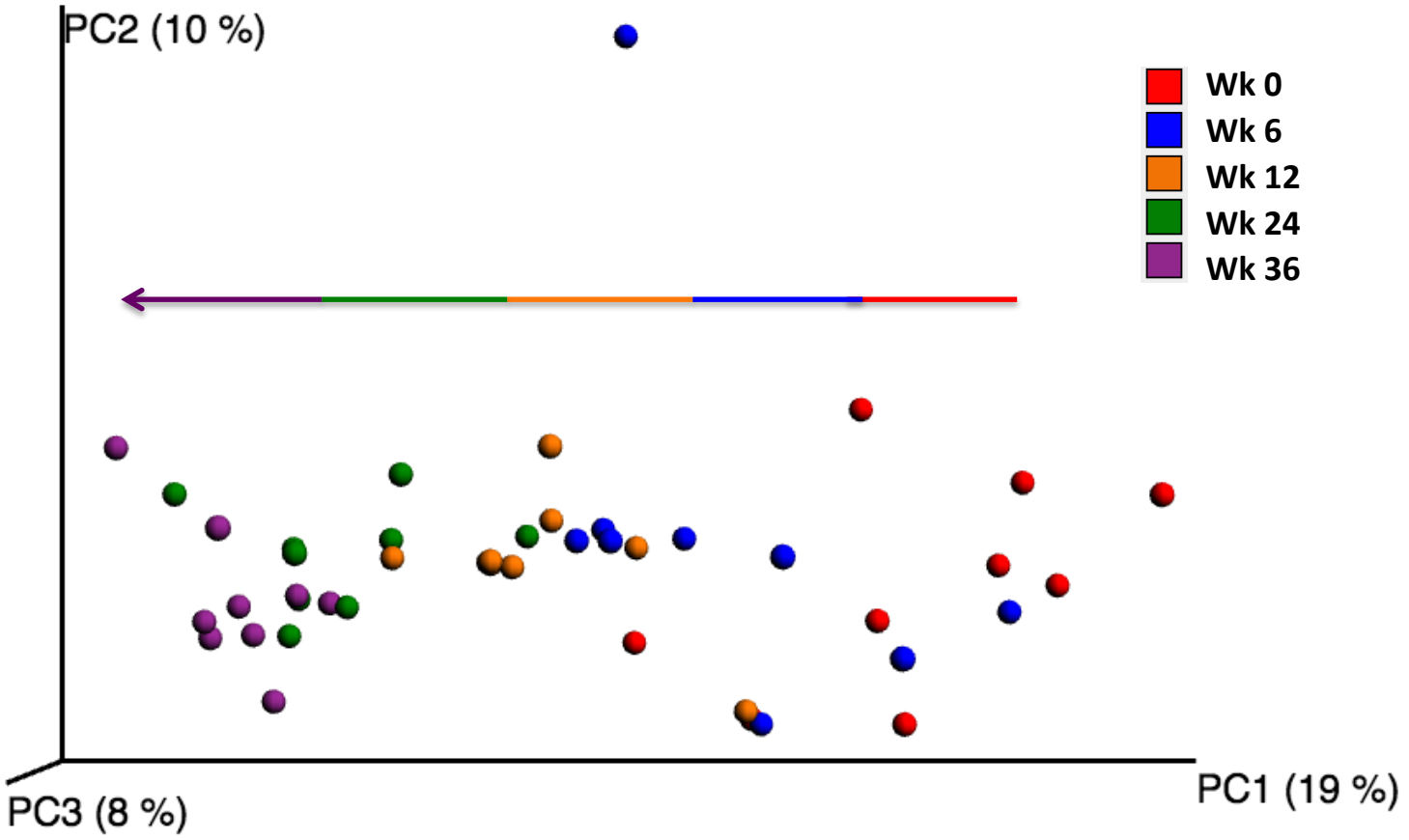
Jia et al., 2011

Diversidade da Microbiota – Durante Desenvolvimento de Obesidade em Gatos



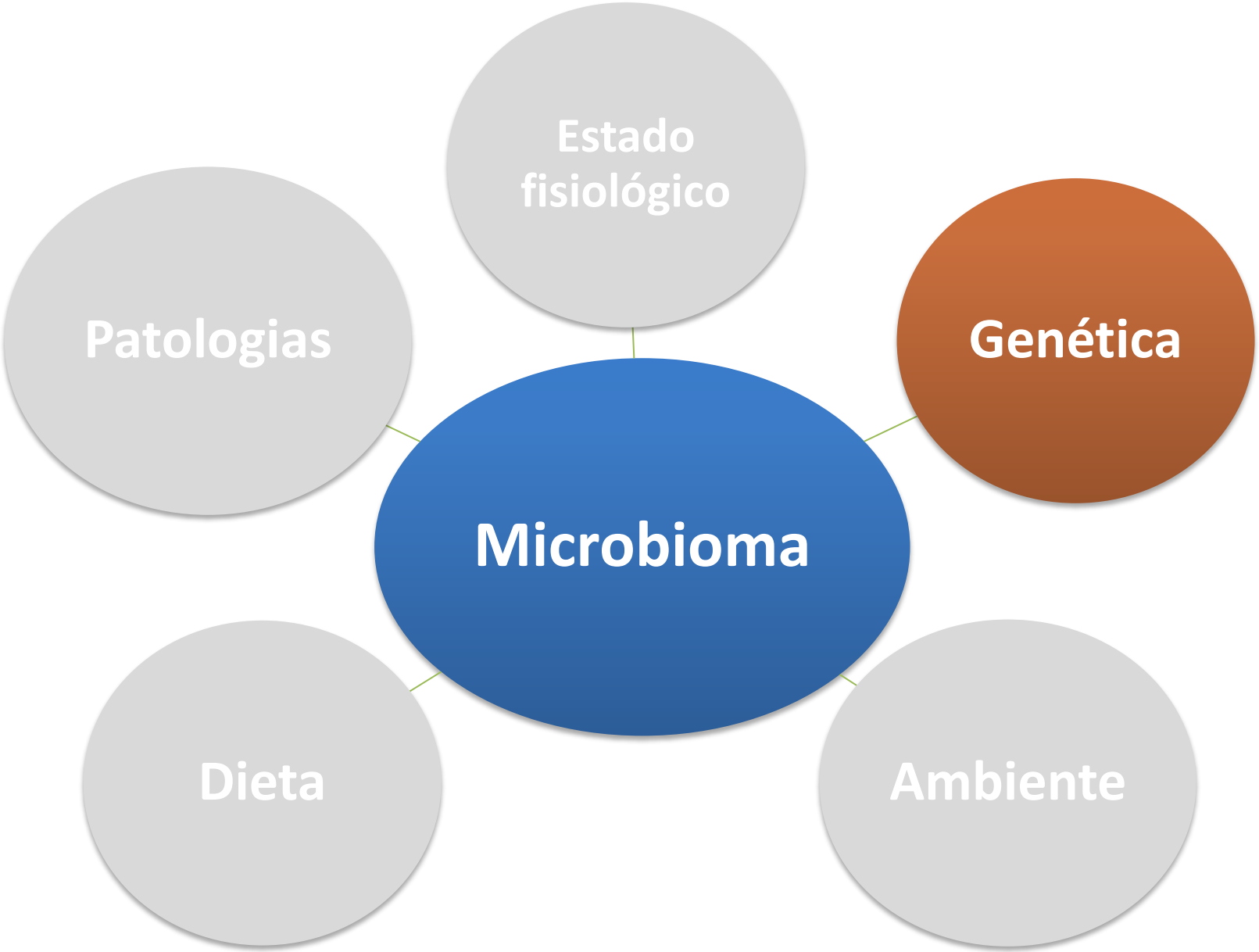
de Godoy et al., unpublished

PCoA– Durante Desenvolvimento de Obesidade em Gatos

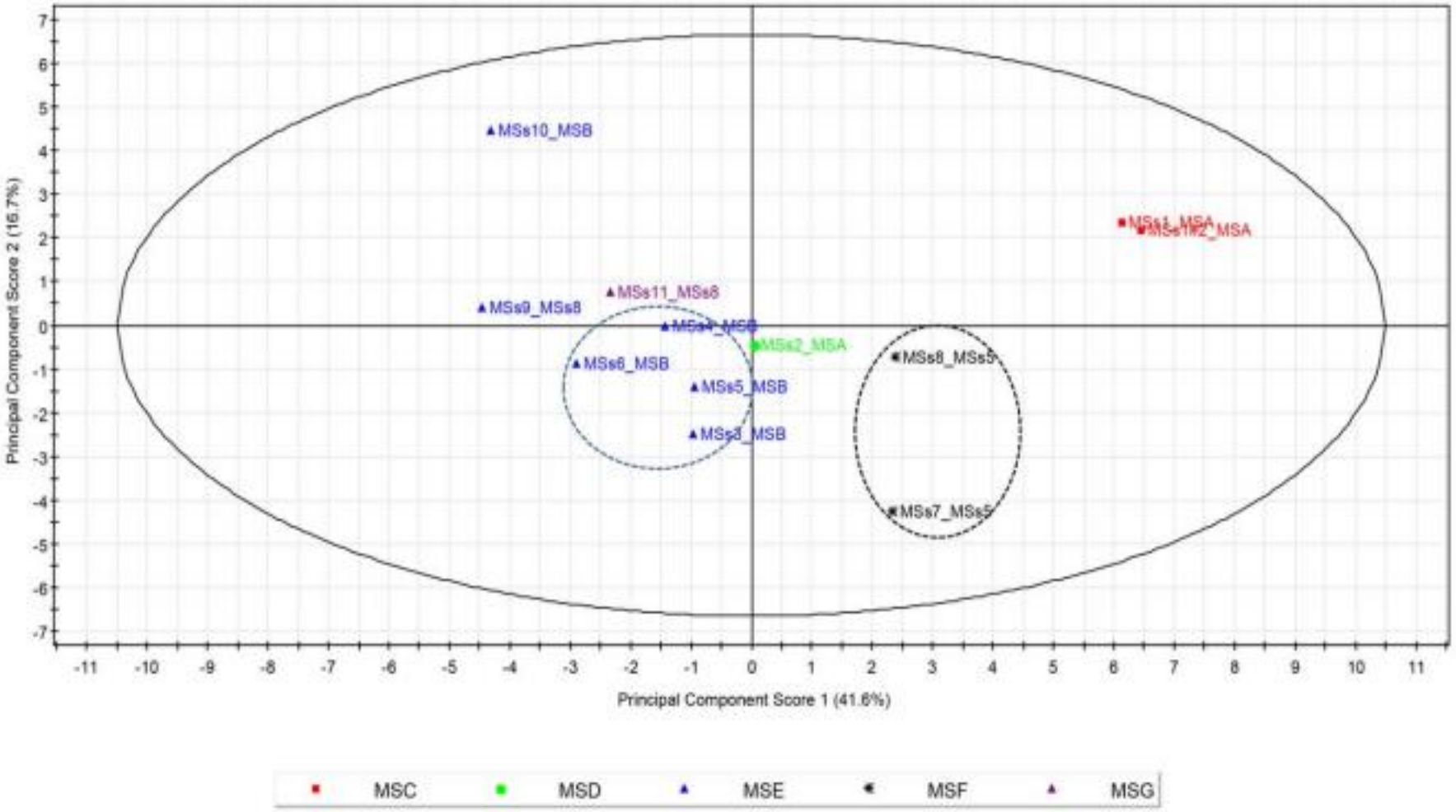


de Godoy et al., unpublished

Modulação do Microbioma

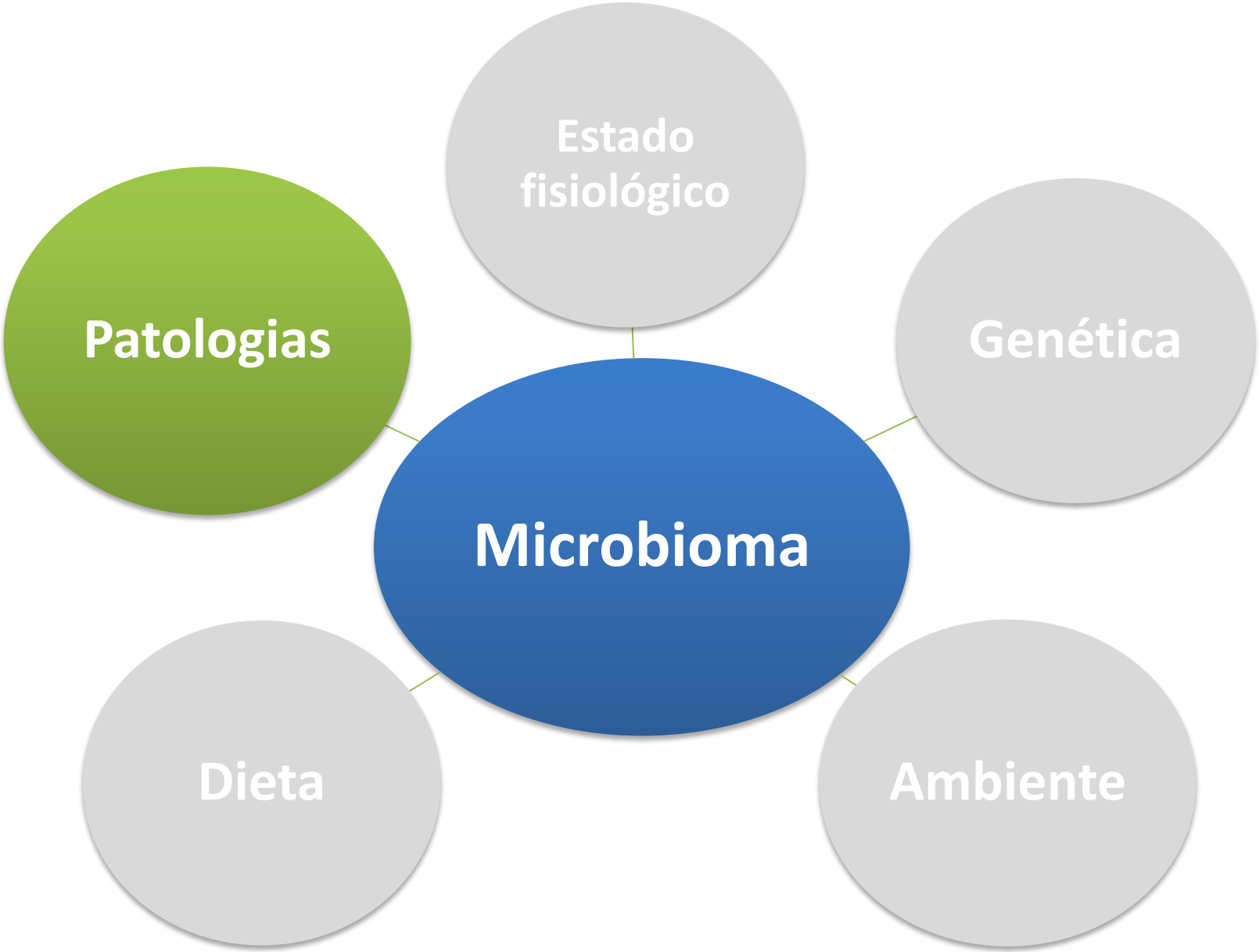


Relação Microbioma- Hospedeiro

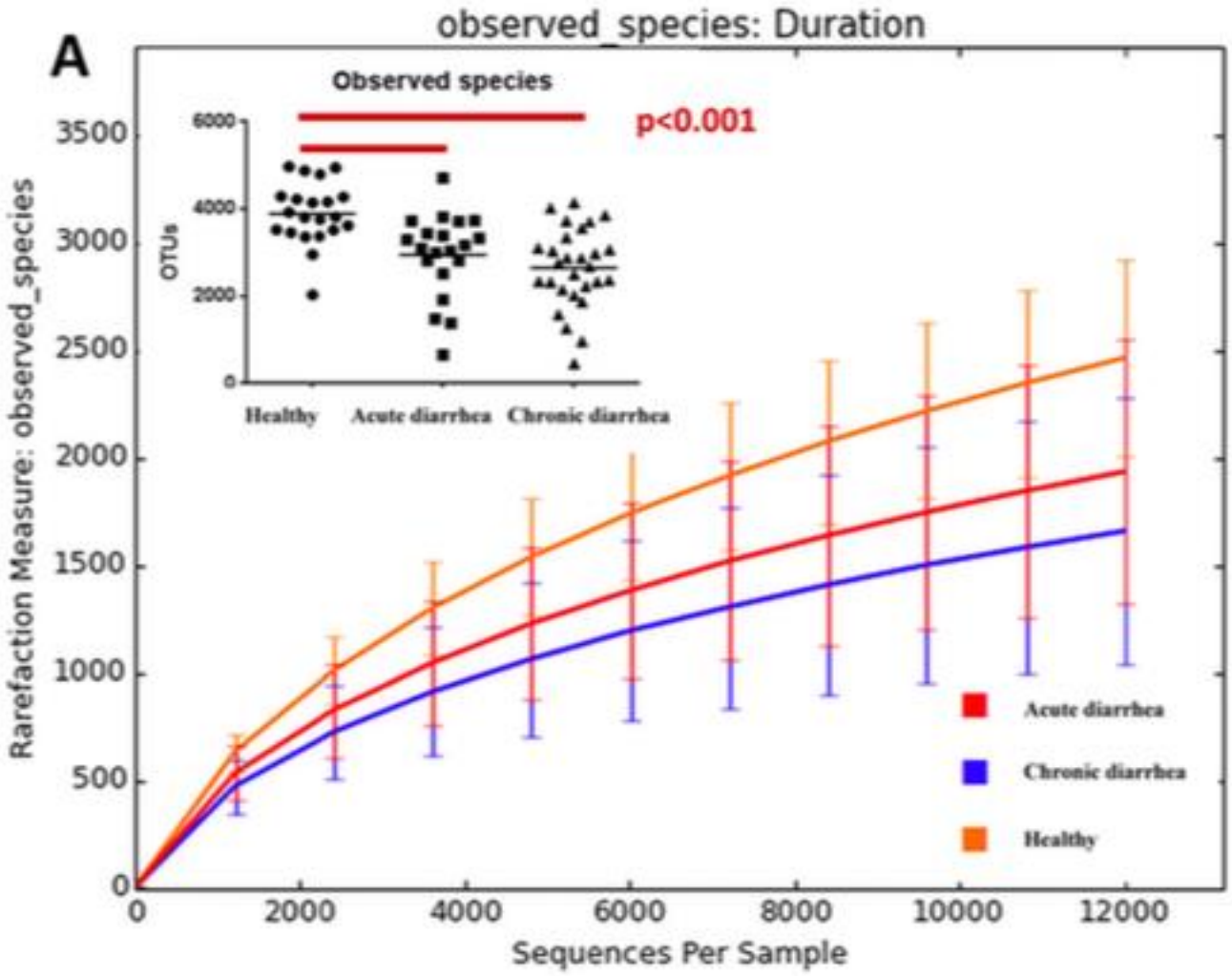


Hand et al., 2013

Modulação do Microbioma

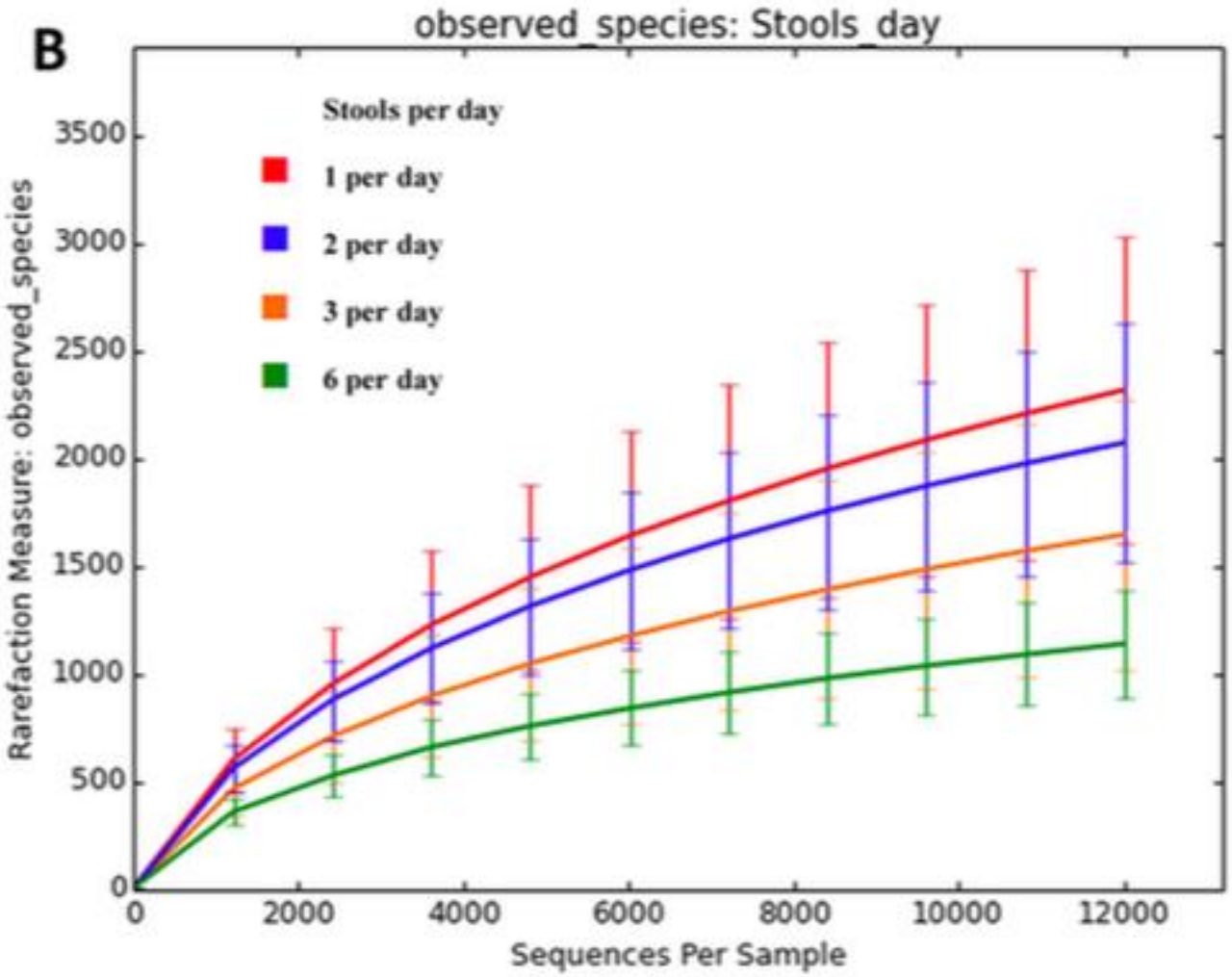


Alterações no Microbioma em Gatos com Diarréia



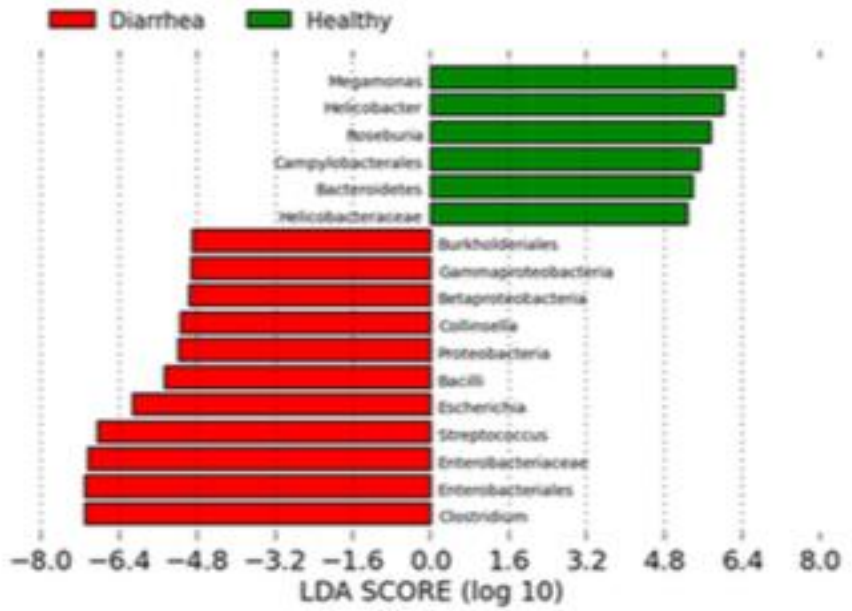
Suchodolski et al., 2015

Frequência de Defecação e Diversidade da Microbiota



Suchodolski et al., 2015

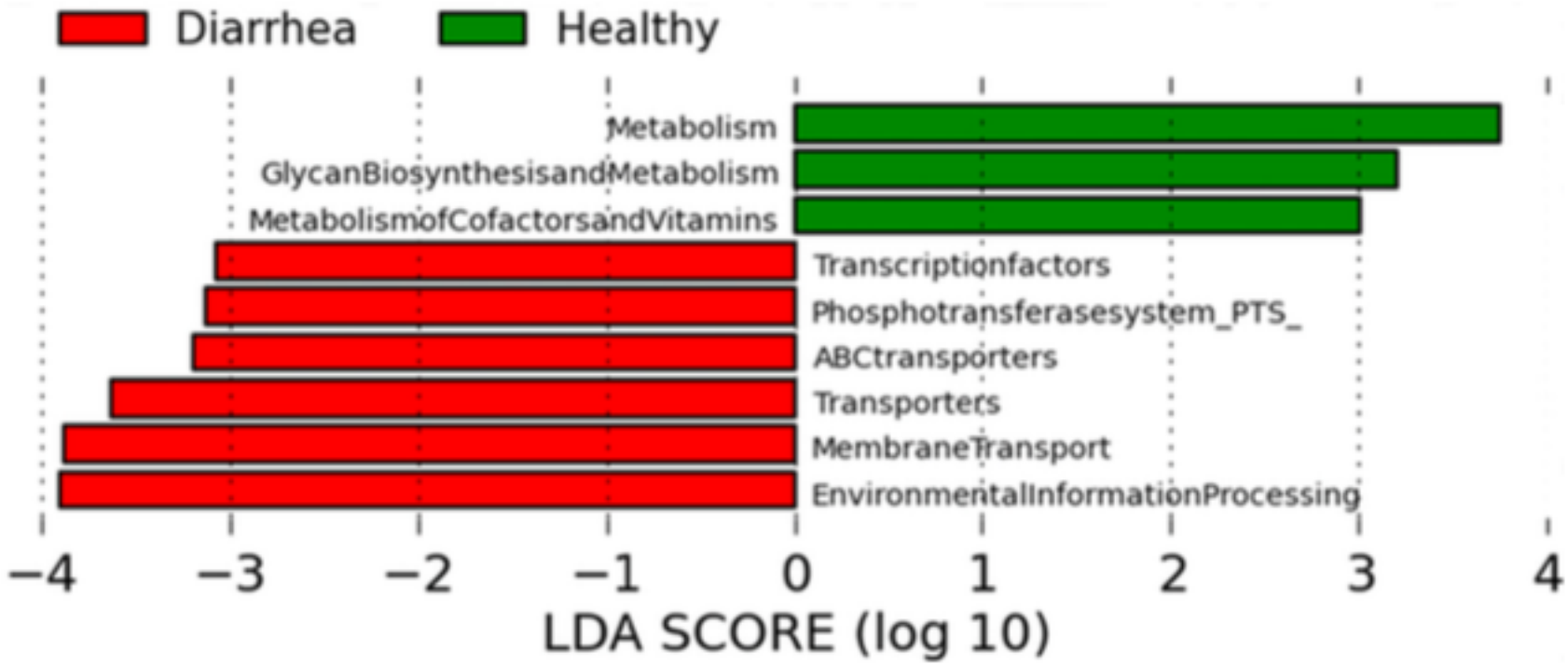
Diferenças da Microbiota em Gatos Saudáveis e Doentes



- Saudáveis
  - ↓ Proteobacteria
    - Delta
  - ↓Bacteroidaceae
  - ↓ Roseburia e Megamonas
- Doentes
  - ↑Proteobacteria
    - Gama e beta
  - ↑ Firmicutes
    - Clostridium

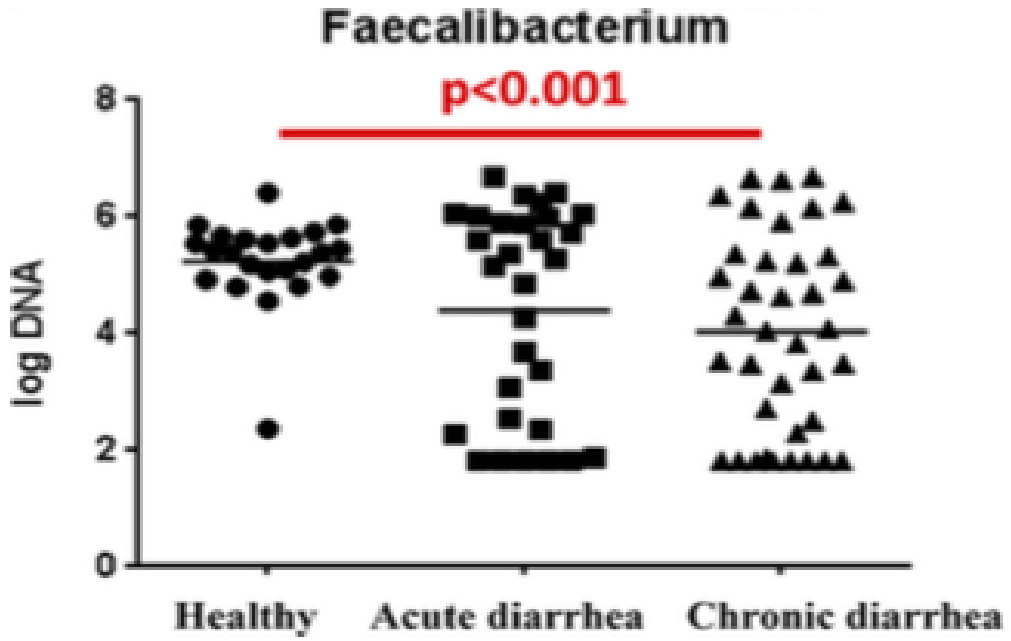
Suchodolski et al., 2015

Diferenças na Função Microbiana em Gatos Saudáveis e Doentes



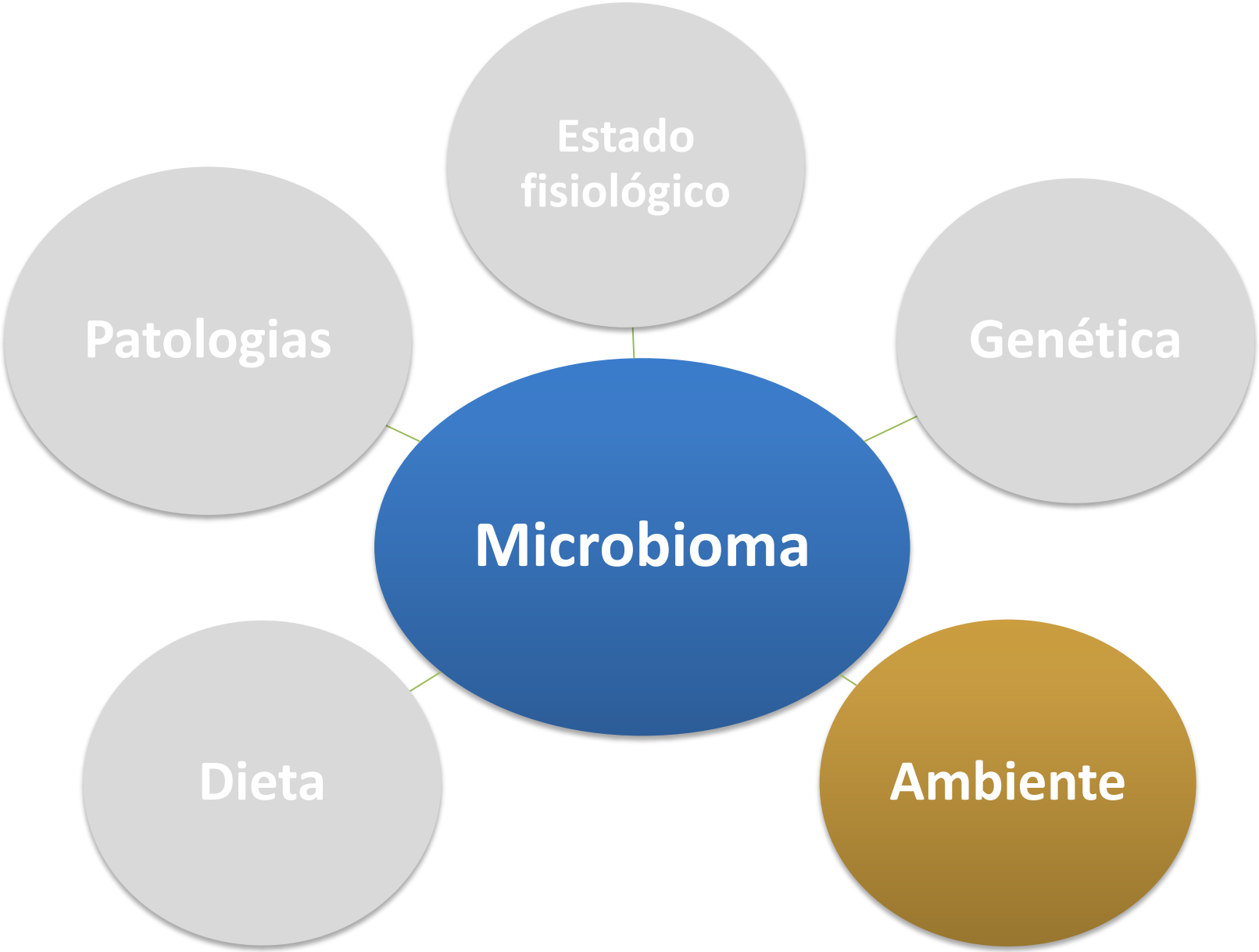
Suchodolski et al., 2015

Diarréia Aguda Diminui Faecalibacterium

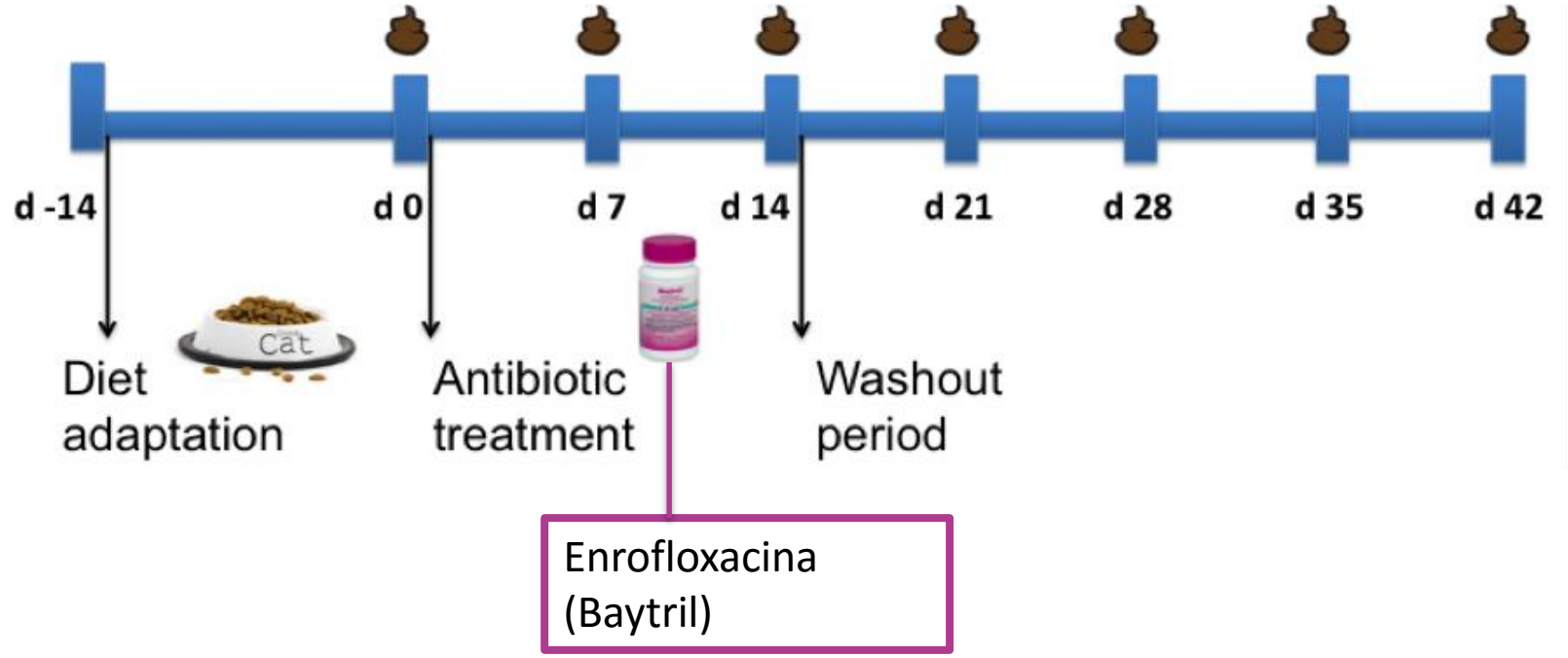


Suchodolski et al., 2015

Modulação do Microbioma

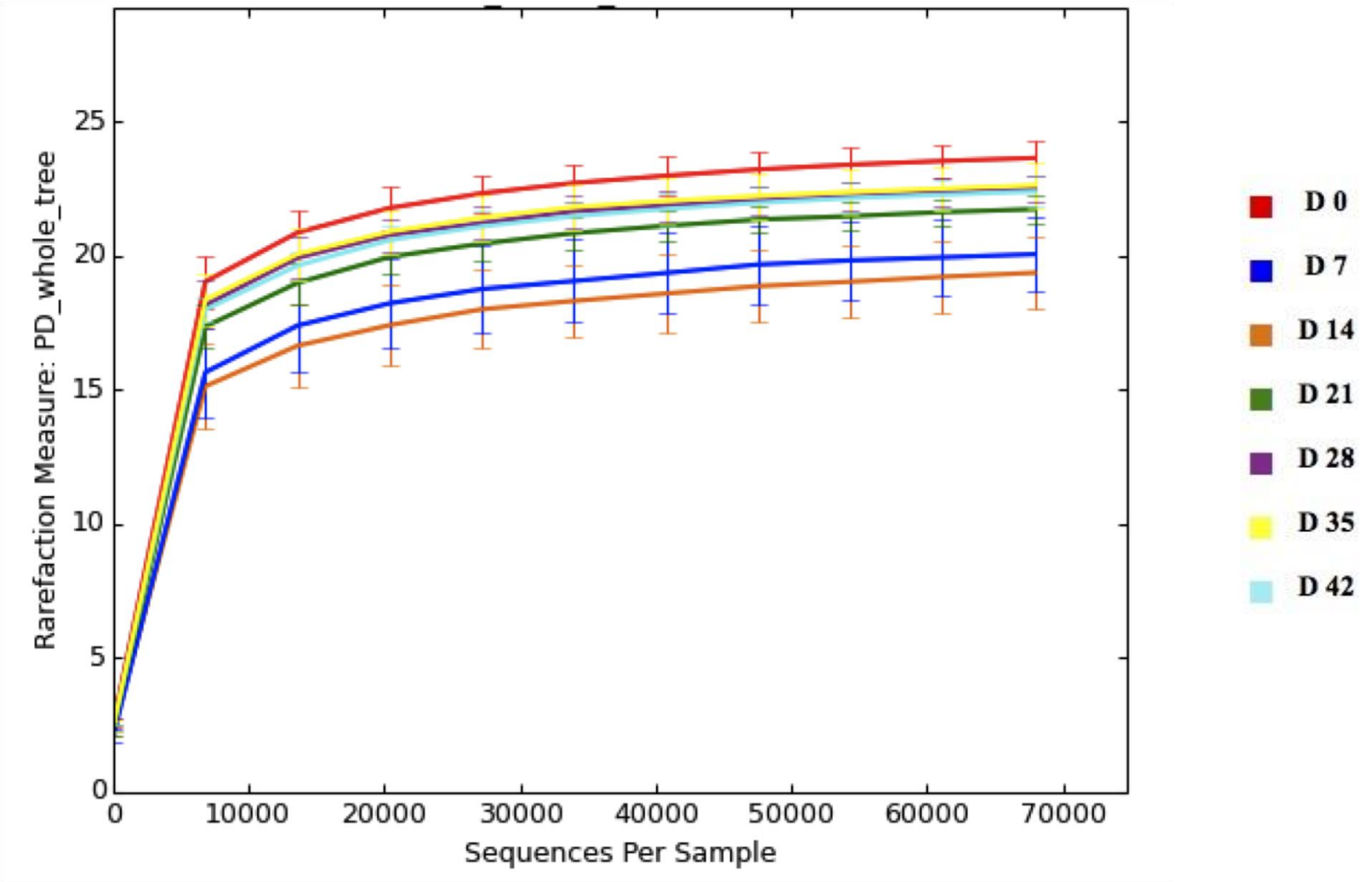


Efeito do Tratamento com Antibiótico na Microbiota Fecal de Felinos



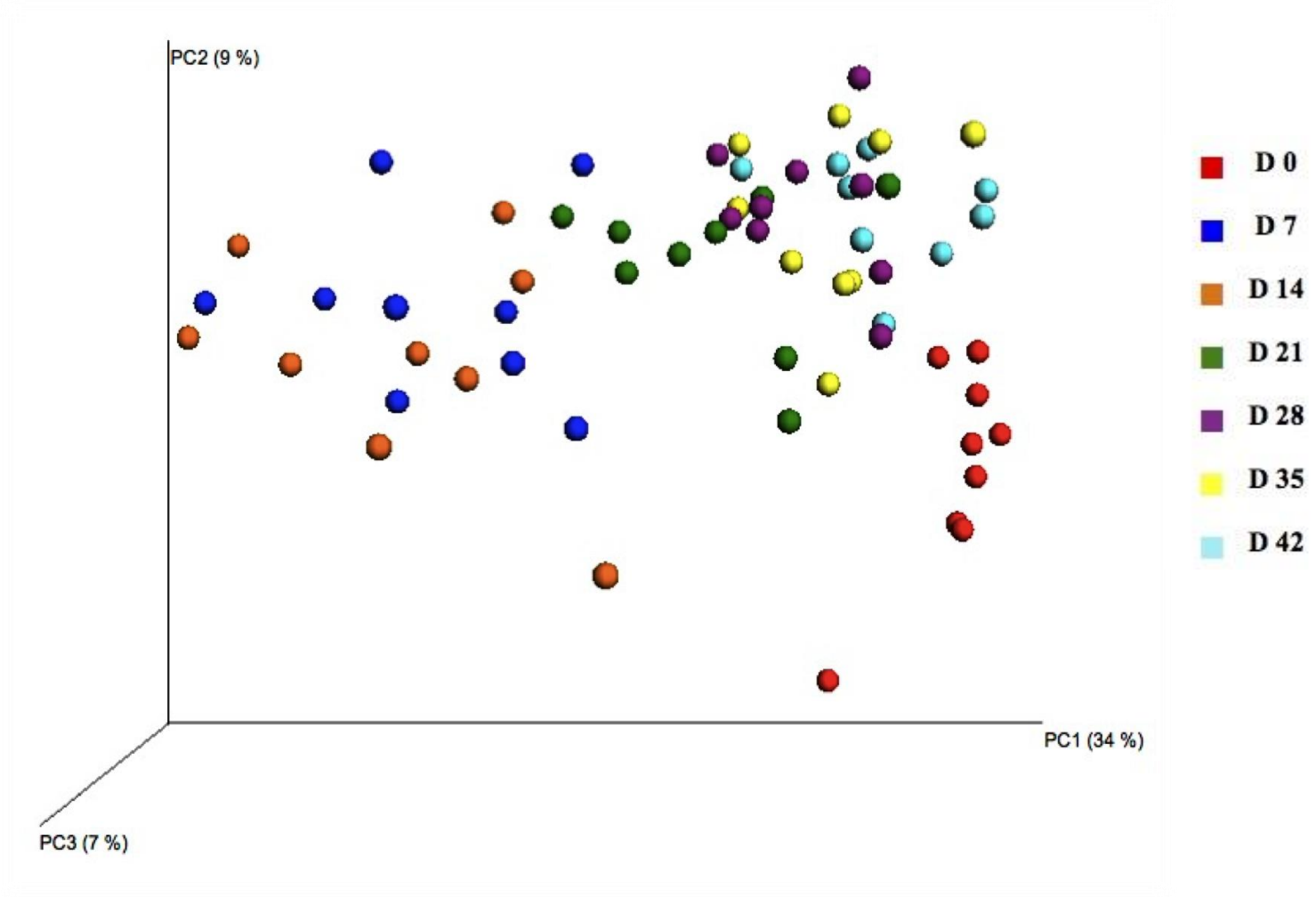
de Godoy et al., unpublished

Diversidade da Microbiota– Antes, Durante e Após Tratamento com Antibiotico



de Godoy et al., unpublished

PCoA - Antes, Durante e Após Tratamento com Antibiotico



de Godoy et al., unpublished

Relação Microbioma- Hospedeiro

- Microbioma de cães e gatos
  - Cavidade oral
  - Trato urinário e genital
  - Epiderme

Relação Microbioma- Hospedeiro

- Microbioma de cães e gatos
  - **Cavidade oral**
    - Periodontite afeta mais de 70% gatos adultos
    - Cães
      - 95-100% gingivite
      - 50-70% periodontite



Lommer and Verstraete, 2001; Harvey et al., 1995

Relação Microbioma- Hospedeiro



PLOS ONE

RESEARCH ARTICLE

A Pyrosequencing Investigation of Differences in the Feline Subgingival Microbiota in Health, Gingivitis and Mild Periodontitis

Stephen Harris[1], Julie Croft[1], Ciaran O'Flynn[1], Oliver Deusch[1], Alison Colyer[1], Judi Allsopp[1], Lisa Milella[2], Ian J. Davis[1]*

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Veterinary Microbiology 181 (2015) 271–282

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

A longitudinal assessment of changes in bacterial community composition associated with the development of periodontal disease in dogs

Corrin Wallis*, Mark Marshall, Alison Colyer, Ciaran O'Flynn, Oliver Deusch, Stephen Harris

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ARTICLE INFO

Article history:
Received: 3 June 2015
Received in revised form: 11
Accepted: 2 September 2015

Keywords:
Canine
Oral
Gingivitis
Microbiota
Microbiome
Periodontitis

PLOS ONE

RESEARCH ARTICLE

Comparison of the Oral Microbiomes of Canines and Their Owners Using Next-Generation Sequencing

Changin Oh, Kunkyu Lee, Yeotaek Cheong, Sang-Won Lee, Seung-Yong Park, Chang-Seon Song, In-Soo Choi, Joong-Bok Lee*

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OPEN ACCESS

Citation: Oh C, Lee K, Cheong Y, Lee S-W, Park S-Y, Song C-S, et al. (2015) Comparison of the Oral Microbiomes of Canines and Their Owners Using Next-Generation Sequencing. PLoS ONE 10(7): e0131468. doi:10.1371/journal.pone.0131468

Editor: Bryan A. White, University of Illinois, UNITED STATES

Received: January 5, 2015

Accepted: June 2, 2015

Published: July 2, 2015

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Abstract

The oral microbiome, which is closely pathogenic oral bacteria, which can b public health considerations. Althoug composition of the canine oral microb and its relationship with that of their o quencing was used to compare the or identify zoonotic pathogens. Pyroseq the 10 samples, representing 57 gene teobacteria (21.6%), Bacteroidetes (9 were the predominant phyla in the hu Actinobacteria (21%), Bacteroidetes ((12.3%) were predominant in the cani human samples were *Streptococcus* *Prevotella* (8.4%), and *Veillonella* (8.1 samples were *Actinomyces* (17.2%), *ium* (11.8), and *Neisseria* (7.2%). The appreciably different, and similarity in correlated with residing in the same h *Porphyromonas canigingivalis*, *Tanne* humans was suspected. The finding the canine oral microbiome may be a

genomeA JournalsASM.org

Draft Genome Sequences of 26 *Porphyromonas* Strains Isolated from the Canine Oral Microbiome

David A. Coil,[a] Alexandra Alexiev,[a] Corrin Wallis,[b] Ciaran O'Flynn,[b] Oliver Deusch,[b] Ian Davis,[b] Alexander Horsfall,[b] Nicola Kirkwood,[b] Guillaume Jospin,[a] Jonathan A. Eisen,[a,c] Stephen Harris,[b] Aaron E. Darling[d]

University of California Davis Genome Center, Davis, California, USA; The Waltham Centre for Pet Nutrition, Melton Mowbray, Leicestershire, United Kingdom; Department of Evolution and Ecology and Department of Medical Microbiology and Immunology, University of California Davis, Davis, California, USA; iThree Institute, University of Technology, Sydney, Australia

We present the draft genome sequences for 26 strains of *Porphyromonas* (*P. canoris*, *P. gulae*, *P. cangingavalis*, *P. macacae*, and 7 unidentified) and an unidentified member of the *Porphyromonadaceae* family. All of these strains were isolated from the canine oral cavity, from dogs with and without early periodontal disease.

Received 10 February 2015 Accepted 3 March 2015 Published 9 April 2015

Citation Coil DA, Alexiev A, Wallis C, O'Flynn C, Deusch O, Davis I, Horsfall A, Kirkwood N, Jospin G, Eisen JA, Harris S, Darling AE. 2015. Draft genome sequences of 26 *Porphyromonas* strains isolated from the canine oral microbiome. Genome Announc. 3(2):e00187-15. doi:10.1128/genomeA.00187-15.

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Address correspondence to Jonathan A. Eisen, jaeisen@ucdavis.edu.

Members of the *Porphyromonas* genus have been shown to be present in periodontal disease in both humans and dogs (1, 2). As part of a larger study on canine periodontal disease (1), a number of *Porphyromonas* strains were isolated, cultured, and sequenced.

The study cohort comprised client-owned pet dogs that presented at a veterinary referral dental clinic (The Veterinary Dental Surgery, Surrey, United Kingdom). Only dogs under anesthetic for routine dental treatment or other noninfectious conditions were included in the study. No dogs were anesthetized solely for the collection of plaque samples. For further details see our previously published work on this cohort (1).

PLOS ONE

RESEARCH ARTICLE

Early Canine Plaque Biofilms: Characterization of Key Bacterial Interactions Involved in Initial Colonization of Enamel

Lucy J. Holcombe*, Niran Patel, Alison Colyer, Oliver Deusch, Ciaran O'Flynn, Stephen Harris

The WALTHAM Centre for Pet Nutrition, Leicestershire, United Kingdom

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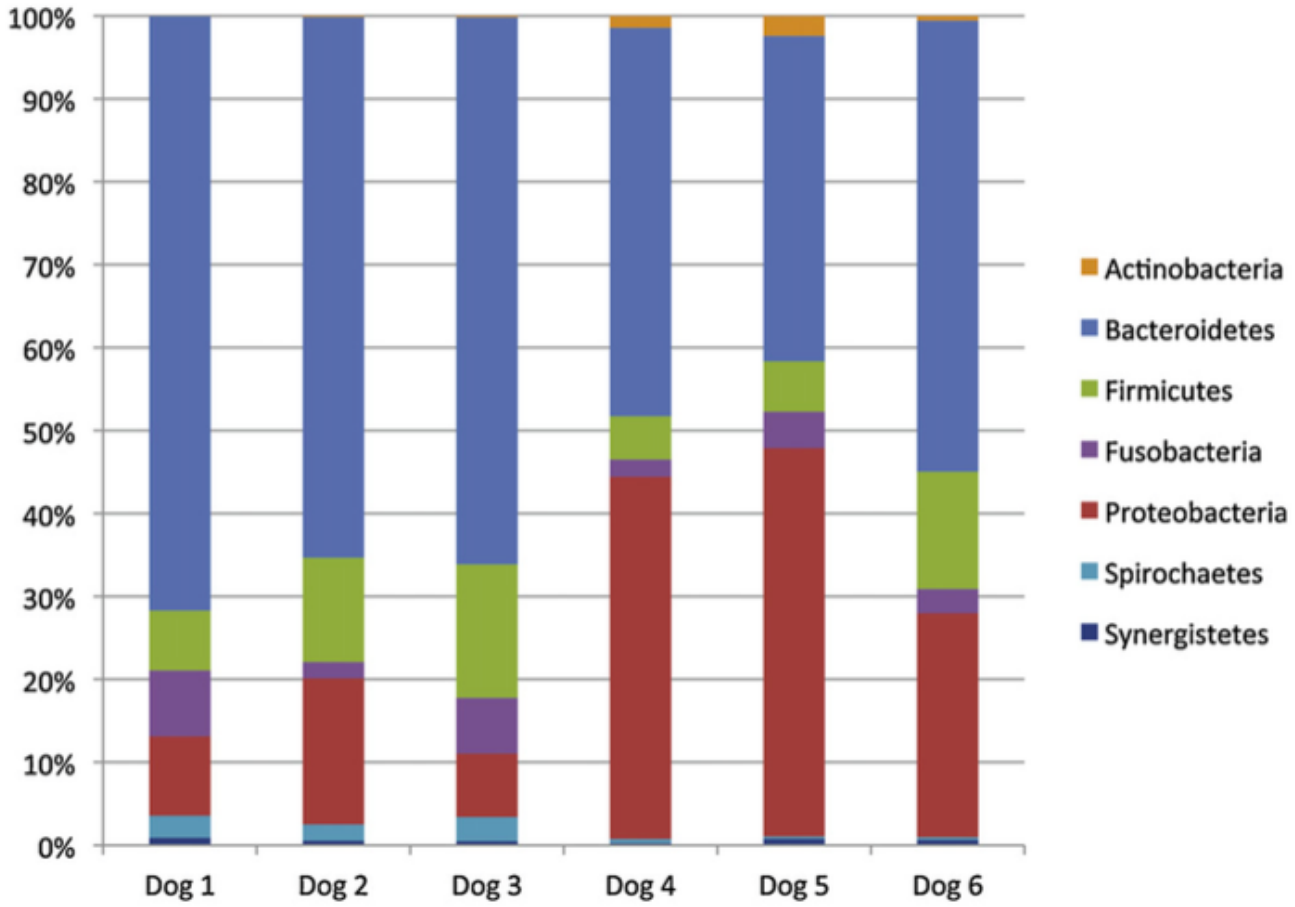
OPEN ACCESS

Citation: Holcombe LJ, Patel N, Colyer A, Deusch O, O'Flynn C, et al. (2014) Early Canine Plaque Biofilms: Characterization of Key Bacterial

Abstract

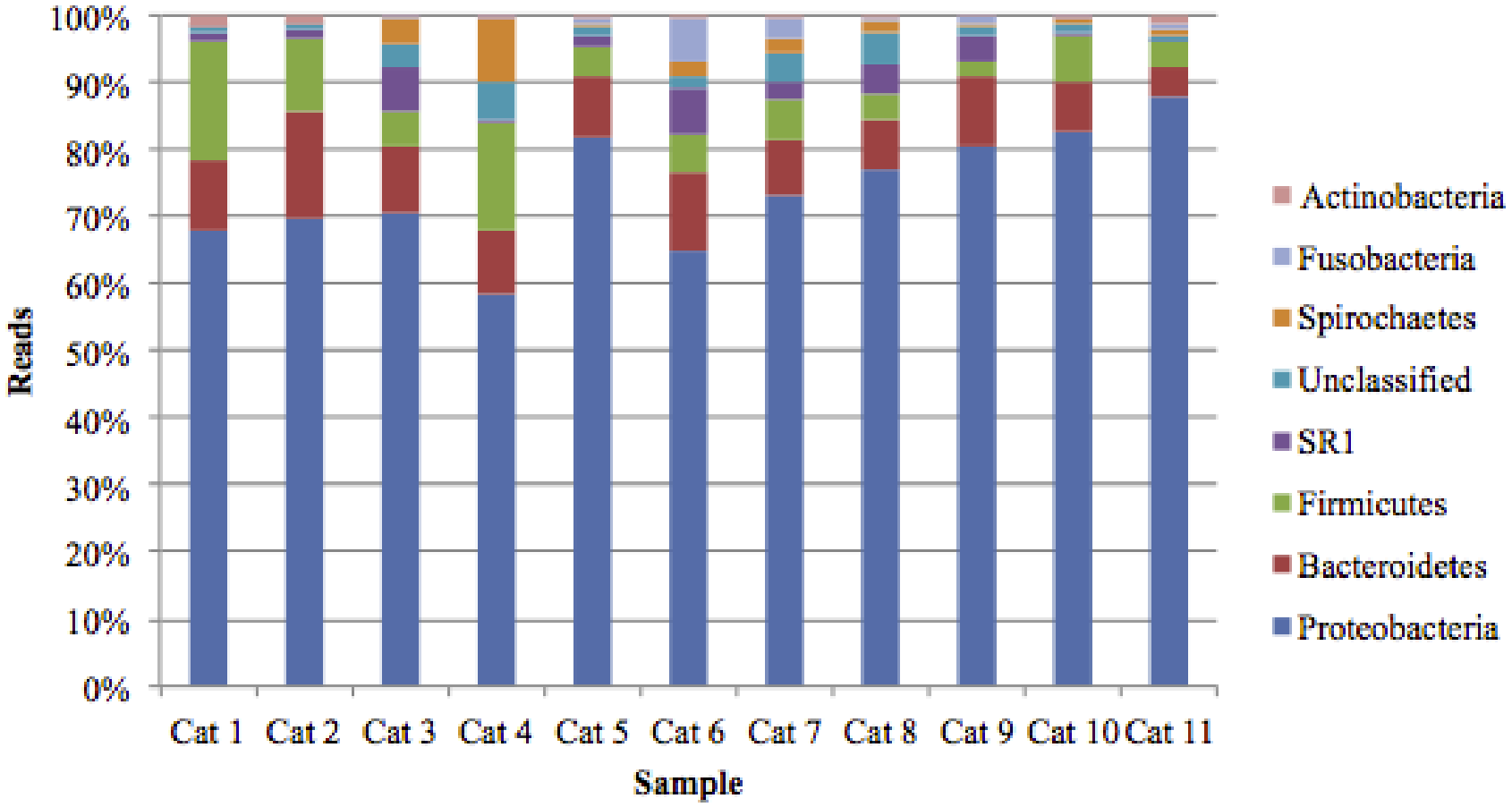
Periodontal disease (PD) is a significant problem in dogs affecting between 44% and 63.6% of the population. The main etiological agent for PD is plaque, a microbial biofilm that colonizes teeth and causes inflammation of the gingiva. Understanding how this biofilm initiates on the tooth surface is of central importance in developing interventions against PD. Although the stages of plaque development on human teeth have been well characterized little is known about how canine plaque develops. Recent studies of the canine oral microbiome have revealed distinct differences between the canine and human oral environments and the bacterial communities they support, particularly with respect to healthy plaque. These differences mean knowledge about the nature of plaque formation in humans may not be directly translatable to dogs. The aim of this study was to identify the bacterial species important in the early stages of canine plaque

Distribuição Relativa do Filo Microbiano Oral – Cães Saudáveis



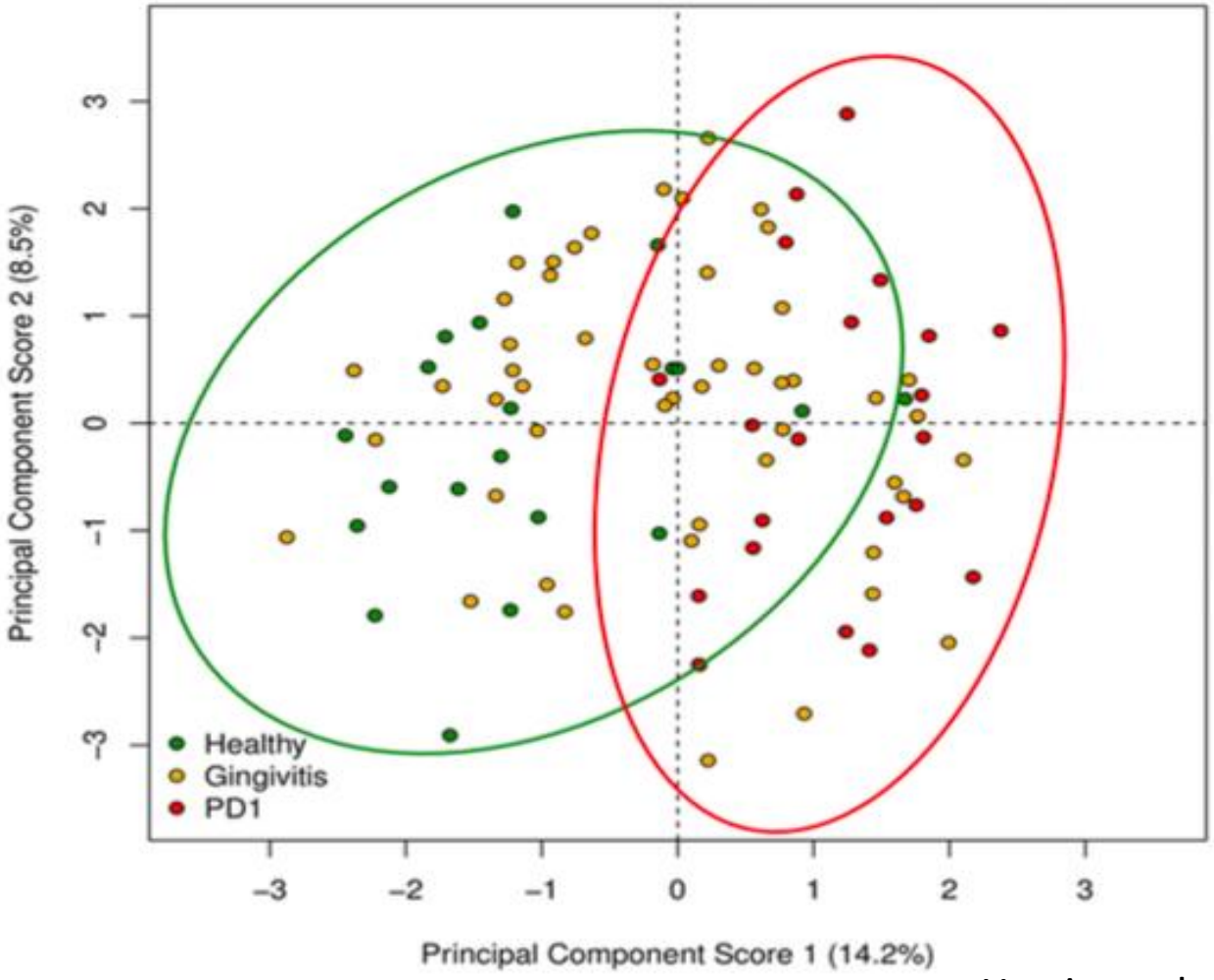
Sturgeon et al., 2013

Distribuição Relativa do Filo Microbiano Oral – Gatos Saudáveis



Sturgeon et al., 2014

PCA: Microbiota Subgengival de Gatos Saudáveis, com Gengivite ou Periodontite em Fase Inicial



Harris et al., 2015

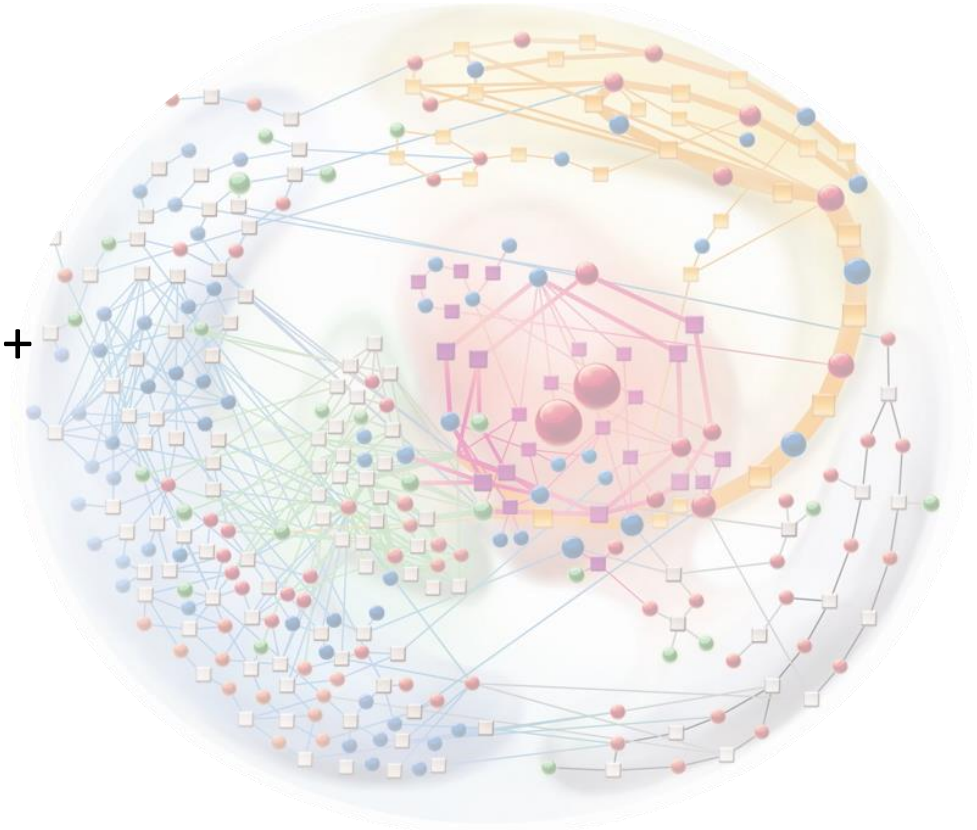
Desafios

- Padronização da tecnologia
  - Facilitar comparação entre estudos
  - Minimizar influência de primers usados
- Caracterização mais detalhada do microbioma
  - Sequenciamento mais profundo
    - Gênero, espécies, estirpes
- Ecosistema dinâmico
  - Estudos longitudinais



Desafios

- Desenvolvimento de plataformas que facilitem a análise de dados e a integração de diferentes bancos de dados
  - Metaboloma + microbioma + transcriptoma



Oportunidades

- Proprietários de cães e gatos
  - Aumentar longevidade
  - Melhorar qualidade de vida
  - Minimizar doenças



Oportunidades

- Cães e gatos → adequado modelos animais pra humanos
  - Semelhante doenças
  - Dividem mesmo ambiente
  - Dieta semelhante

- Apresentam várias vantagens:
  - Controle da dieta e ambiente
  - Facilita estudos longitudinais

Próximos Capítulos…



- Fatores que levam a microbiota a resistir e (ou) se recuperar de perturbações

- Estratégias para manipulação da microbiota/ microbioma → saúde

Próximos Capítulos…

- Expandir conhecimento da interação microbioma-hospedeiro em diferentes partes do corpo



Futuro


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MICROBIOTA
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